DCPP (5-Chloro-2-(4-chlorophenoxy)-phenol)

Biocide for Use as Human hygiene biocidal product (PT 1)
Private area and public health area disinfectant and other biocidal products (PT 2)
Food and feed area disinfectant (PT 4)

Dossier According to Directive 98/8/EC

Document III-A

Data on the Active Substance

Section A1 Applicant

Annex Point IIA, I 1

				Official use only
1.1	**Applicant**	Company name:	BASF SE (formerly Ciba Spezialitätenchemie Grenzach GmbH)	
		Contact name:	[redacted]	
		Address:	Carl-Bosch Strasse D-67056 Ludwigshafen, Germany	
		Telephone:	[redacted]	
		E-Mail:	[redacted]	
1.2	**Manufacturer of Active Substance** (if different)	[redacted] (Reference: [redacted] For manufacturer of the active substance please refer to the confidential part of the dossier.		x
1.3	**Manufacturer of Product(s) (if different)**	Please refer to Document IIIB, Section 1.		

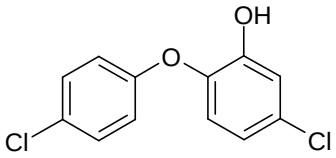
Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2010
Materials and methods	-
Conclusion	1.2: According to 98/8/EC, Article 19, confidentiality shall not in any case apply to the name and address of the active substance manufacturer. The active substance as produced by [redacted]
Reliability	n.a.
Acceptability	Acceptable with the amendments given above.
Remarks	-

Section A2 Identity of Active Substance - June 2014

Annex point IIA, II 2

Subsection (Annex Point)				Official use only
2.1	**Common name (IIA, II)**	DCPP		
2.2	**Chemical name (IIA, II 2.2)**	IUPAC name: CAS name: Other name:	5-Chloro-2-(4-chlorophenoxy)-phenol Phenol, 5-chloro-2-(4-chlorophenoxy)- 2-Hydroxy-4,4'-dichlorodiphenylether	
2.3	**Manufacturer´s development code number(s) (IIA, II 2.3)**	[redacted]		
2.4	**CAS No and EC numbers (IIA, II 2.4)**			
2.4.1	**CAS-No**	3380-30-1		
	Isomer 1	Not relevant		
	Isomer n	Not relevant		
2.4.2	**EC-No**	429-290-0		
	Isomer 1	Not relevant		
	Isomer n	Not relevant		
2.4.3	**Other**	Not allocated		
2.5	**Molecular and structural formula, molecular mass (IIA, II 2.5)**			
2.5.1	**Molecular formula**	$C_{12}H_8Cl_2O_2$		
2.5.2	**Structural formula**			
2.5.3	**Molecular mass**	255.1 g/mol		
2.6	**Method of manufacture of the active substance (IIA, II 2.6)**	[confidential information]		
2.7	**Specification of the purity of the active substance, as appropriate (IIA, II 2.7)**	DCPP has a specified minimal purity of 99% (990 g/kg). The specification limit is ≥99%.		x

Section A2 Identity of Active Substance - June 2014

Annex point IIA, II 2

2.8	**Identity of impurities and additives, as appropriate (IIA, II 2.8)**	[confidential information]
2.8.1	**Isomeric composition**	Not applicable since the molecule of the active substance has no centre of asymmetry.
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA, II 2.9)**	Not applicable as the active substance has no natural origin.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
Date	August 2014
Materials and methods	-
Conclusion	Acceptable with the amendments given below.
Reliability	1
Acceptability	Acceptable with the amendments given below.
Remarks	2.7 Specification of the purity of the active substance: The minimum degree of purity of DCPP, calculated as (AV – 3 SD) amounts to 99.5%w/w

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

Official use only

Subsection

2.10.1 Human exposure towards active substance

2.10.1.1 Production

i) Description of process	[redacted]
ii) Workplace description	[redacted]
iii) Inhalation exposure	[redacted]
iv) Dermal exposure	[redacted]

2.10.1.2 Intended use(s)

Product types 1, 2, and 4

1. Professional Users

i) Description of application process	PT1: Hand soap: 1-min application followed by water rinse
	PT2: Surface disinfection: wiping and/or mopping
	PT4: Manual dishwashing
ii) Workplace description	PT1: Hospital or medical practice; consumer use is also intended
	PT2: Private area or public health area
	PT4: Restaurant or private household
iii) Inhalation exposure	PT1: negligible
	PT2: negligible
	PT4: negligible
iv) Dermal exposure	PT1: 0.0719 mg/kg/day
	PT2: 0.0022 mg/kg/day
	PT4: 6.6×10^{-4} mg/kg/day

2. Non-professional Users including the general public

(i) via inhalational contact	PT1: negligible
	PT2: negligible
	PT4: negligible
(ii) via skin contact	PT1: 0.0719 mg/kg/day
	PT2: 0.0045 mg/kg/day
	PT4: 6.6×10^{-4} mg/kg/day

Section A2.10 Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

Annex Point IIA2.10

(iii) via drinking water	Not relevant
(iv) via food	PT4: 2.8×10^{-7} mg/kg/day
(v) indirect via environment	Not relevant

2.10.2 Environmental exposure towards active substance

2.10.2.1 Production

(i) Releases into water	Not relevant, since the production of active substance takes place outside European Union.
(ii) Releases into air	Not relevant, since the production of active substance takes place outside European Union.
(iii) Waste disposal	Not relevant, since the production of active substance takes place outside European Union.

2.10.2.2 Intended use(s)

	Product types 1, 2, and 4
Affected compartment(s):	All PTs: STP, surface water/sediment, soil, biota
Predicted concentration in the affected compartment(s)	
STP:	PT1: 0.16 / 0.0004 µg/L (Tier 1 / 2)
	PT2: 17.75 / 0.07 µg/L (Tier 1 / 2)
	PT4: 0.15 / 0.003 µg/L (Tier 1 / 2)
surface water	PT1: 0.016 / 0.00004 µg/L (Tier 1 / 2)
	PT2: 1.77 / 0.007 µg/L (Tier 1 / 2)
	PT4: 0.02 / 0.0003 µg/L (Tier 1 / 2)
sediment	PT1: 0.5034 / 0.0013 µg/kg (Tier 1 / 2)
	PT2: 55.76 / 0.22 µg/kg (Tier 1 / 2)
	PT4: 0.48 / 0.009 µg/kg (Tier 1 / 2)
air	not relevant
soil	PT1: 0.0002 / 1.763×10^{-6} mg/kg (Tier 1 / 2)
	PT2: 22.37 / 0.31 mg/kg (Tier 1 / 2)
	PT4: 0.19 / 0.013 mg/kg (Tier 1 / 2)

Section A2.10
Annex Point IIA2.10

Exposure data in conformity with Annex VIIA to Council Directive 92/32/EEC (OJ No L, 05.06.1992, p. 1) amending Council Directive 67/548/EEC

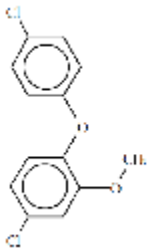
groundwater	not relevant
biota	PT1: 1.23 / 0.003 µg/kg (Tier 1 / 2)
	PT2: 136.35 / 0.54 µg/kg (Tier 1 / 2)
	PT4: 1.17 / 0.023 µg/kg (Tier 1 / 2)

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2012
Materials and methods	-
Conclusion	2.10.1 Human exposure towards active substance and 2.10.2 Environmental exposure towards active substance This document represents the applicant's exposure assessment. In the course of detailed evaluation content of the exposure assessment was amended by the competent authority. The acceptable intended use is as given in Doc. II-B chapter 3. The accepted human exposure assessment is as given in Doc. II-B chapter 4. The accepted environmental exposure assessment is as given in Doc. II-B chapter 5.
Reliability	n.a.
Acceptability	Acceptable with the amendments/replacements given above
Remarks	n.a.

Section A2 Identity of metabolite Methyl DCPP

Annex point IIA, II 2

Official use only

Subsection (Annex Point)			
2.1	**Common name (IIA, II)**	Methyl-DCPP	
2.2	**Chemical name (IIA, II 2.2)**	IUPAC name: CAS name: Other names:	Benzene, 4-chloro-1-(4-chlorophenoxy)-2-methoxy 4, 4,- dichloro-2'-methoxydiphenyl-ether Methoxy-diclosan, 2-methoxy-4,4'dichlorophenylether
2.3	**Manufacturer´s development code number(s) (IIA, II 2.3)**	[redacted]	
2.4	**CAS No and EC numbers (IIA, II 2.4)**		
2.4.1	**CAS-No**	4640-07-7	
	Isomer 1	Not relevant	
	Isomer n	Not relevant	
2.4.2	**EC-No**	Not allocated	
	Isomer 1	Not relevant	
	Isomer n	Not relevant	
2.4.3	**Other**	Not allocated	
2.5	**Molecular and structural formula, molecular mass (IIA, II 2.5)**		
2.5.1	**Molecular formula**	$C_{13}H_{10}Cl_2O_2$	
2.5.2	**Structural formula**		
2.5.3	**Molecular mass**	269.13 g/mol	
2.6	**Method of manufacture of the active substance (IIA, II 2.6)**	Not relevant as the substance is a degradation metabolite and is not produced as an active substance for entry into market.	

Section A2 Identity of metabolite Methyl DCPP

Annex point IIA, II 2

2.7	**Specification of the purity of the active substance, as appropriate (IIA, II 2.7)**	Me-DCPP has a specified minimal purity of >99% (990 g/kg), as referenced in the accompanying references.	x
2.8	**Identity of impurities and additives, as appropriate (IIA, II 2.8)**	Not relevant	
2.8.1	**Isomeric composition**	Not applicable since the molecule of the active substance has no centre of asymmetry.	
2.9	**The origin of the natural active substance or the precursor(s) of the active substance (IIA, II 2.9)**	Not applicable as the active substance has no natural origin.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2013
Materials and methods	-
Conclusion	Agree with applicants version
Reliability	-
Acceptability	Acceptable
Remarks	-

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA, III 3.1)								
3.1.1 Melting point	OECD guideline 102 (differential thermal calorimeter)	Purity: > 99% Specification: min. 96%	73.6 °C ± 0.3 °C	–	Y	1	[redacted]	x
3.1.2 Boiling point	OECD guideline 103 (capillary tester)	Purity: > 99% Specification: min. 96%	359.3 °C ± 0.3 °C	–	Y	1	T[redacted]1999b	x
3.1.3 Bulk density/ relative density								
Relative density	OECD guideline 109 (gas comparison pycnometer)	Purity: > 99% Specification: min. 96%	1.47 at 20.1 °C		Y	1	[redacted]1999c	x
Bulk density	CIPAC MT 186	Purity: 99.97% Specification: min. 96%	Pour density: 0.447 g/mL Tap density: 0.613 g/mL	–	N	2	[redacted] 2007	x
3.2 Vapour pressure and Henry´s Law Constant (IIA, III 3.2)								

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Vapour pressure	OECD guideline 104 (calculated using the modified Watson correlation)	Purity: > 99% Specification: min. 96%	$1.2*10^{-06}$ Pa at 25 °C	The vapour pressure can be calculated if it is expected to be so small that the value is below the measurement range of an experimental determination.	N	2	T[redacted]1998	x
Henry's Law Constant	Calculation based on QSAR methods using computer program from US EPA (EPIWIN software: HENRYWIN v3.10)	–	Results at 25 °C: $6.82*10^{-04}$ $Pa*m^3*mol^{-1}$ (Bond method) $2.53*10^{-03}$ $Pa*m^3*mol^{-1}$ (Group method)	–	N	2	[redacted]2007	x
3.3 Appearance (IIA, III 3.3)								
3.3.1 Physical state	Visual inspection	Purity: 99.97% Specification: min. 96%	Crystalline powder	–	Y	1	[redacted] 2007a	x
3.3.2 Colour	Visual inspection	Purity: 99.97% Specification: min. 96%	White (pale grey)	–	Y	1	[redacted] 2007a	x
3.3.3 Odour	Olfactory inspection	Purity: 99.97% Specification: min. 96%	Slightly smelling like phenols	–	Y	1	[redacted]l, 2007a	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.4 Absorption spectra (IIA, III 3.4)								
UV/VIS	OECD guideline 101	Purity: 99% Specification: min. 96%	DCPP was identified by UV/VIS-spectrum. Acetonitrile was used as solvent. Maximum at 277 nm; absorbance = 0.9632; ε = 14.5	–	Y	1	[redacted] 1999	x
IR	The test was performed according to internal standard operation procedures.	Purity: 99% Specification: min. 96%	DCPP was identified by FTIR-spectrum using a KBR-pellet.	–	Y	1	[redacted]1999	x
NMR	The test was performed according to internal standard operation procedures.	Purity: 99% Specification: min. 96%	DCPP was identified by ^{1}H-NMR spectrum. $CDCl_3$ was used as solvent. The NMR-signals correspond to the structure of the substance.	–	Y	1	[redacted], 1999	x
MS	The test was performed according to internal standard operation procedures.	Purity: 99.97% Specification: min. 96%	DCPP was identified by MS spectrum.	–	Y	1	[redacted] 2007a	x
3.5 Solubility in water (IIA, III 3.5)	OECD guideline 105 (column elution method)	Purity: > 99% Specification: min. 96%	19.5 mg/L at 20 °C and pH 5-6	–	Y	1	[redacted]1999d	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
	HPLC-UV	Purity: 99.97% Specification: min. 96%	Results at pH 5: 6.3 mg/L at 10 °C 10.0 mg/L at 20 °C 14.7 mg/L at 30 °C	The solubility of DCPP increases with temperature. At higher pH-values the solubility of DCPP is controlled by the pH-value.	Y	1	[redacted] 2007a [redacted] 2007a and [redacted], 2008	x
3.6 Dissociation constant (-)	OECD guideline 112	Purity: 99.97% Specification: min. 96%	pKa = 9.49 ± 0.06 at 20 °C (mean of two determinations of a 0.01 molar solution of DCPP in ethanol/water)	The determination of the dissociation constant was completed using ethanol, as the active substance is not or not sufficiently soluble in water. The usage of ethanol is permitted according to OECD guideline 112.	Y	1	[redacted] 2007a and 2007c and [redacted] 2007a	x
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III 1)	HPLC-UV	Purity: 99.97% Specification: min. 96%	Solubility in n-hexane: 8.7 g/kg at 10 °C 18.6 g/kg at 20 °C 27.0 g/kg at 30 °C Solubility in n-octanol: 36.8 g/kg at 10 °C 43.7 g/kg at 20 °C 51.4 g/kg at 30 °C	The solubility of DCPP increases with temperature.	Y	1	[redacted] 2007, [redacted] 2007a and [redacted] 2007a	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III 2)	–	–	–	The active substance as manufactured does not include an organic solvent. Therefore no study was performed.	–	–	–	x
3.9 Partition coefficient n-octanol/water (IIA, III 3.6)	OECD guideline 117 (HPLC)	Purity: > 99% Specification: min. 96%	Log Pow = 3.7 at 20 °C	–	Y	1	[redacted]1999e	x
	Calculation	Purity: 99.97% Specification: min. 96%	Log Pow = 4.8 at 10 °C Log Pow = 4.6 at 20 °C Log Pow = 4.5 at 30 °C	–	Y	1	[redacted]2007, [redacted]l, 2007a and [redacted]k, 2007a	x
3.10 Thermal stability, identity of relevant breakdown products (IIA, III 3.7)	OECD guideline 113 (DCS and TGA measurements)	Purity: 99.97% Specification: min. 96%	DCS: The sample shows, neither under air or nitrogen, any thermal effect other than its glass transition at approx. 49 °C and its melting point at approx. 75 °C. TGA: The observed slight loss in weight observed under air as well as under argon can be attributed to the release of some residual water traces.	Based on DCS and TGA measurements, it can be concluded that the active substance is stable between 30 and 150 °C.	Y	1	[redacted], 2007b	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III 3.8)								
Flammability	EC method A.10, A.12 and A.13	Purity: 99.97% Specification: min. 96%	DCPP is not highly flammable. The appraisal of the molecular structure indicates no risk with respect to pyrophoric properties or the potential of evolving flammable gases in contact with water or humid air.	–	Y	1	[redacted] 2007a	x
Auto-flammability	EC method A.16	Purity: 99.97% Specification: min. 96%	DCPP is not auto-flammable.	–	Y	1	[redacted] 2007b	x
3.12 Flash-point (IIA, III 3.9)	–	–	–	Not performed because the active substance is solid.	–	–	–	x
3.13 Surface tension (IIA, III 3.10)	OECD guideline 115 (ring tensiometer)	Purity: > 99% Specification: min. 96%	65 mN/m at 19.7 °C	DCPP is not surface active.	Y	1	[redacted], 1999f	x
3.14 Viscosity (-)	–	–	–	Not performed because the active substance is solid.	–	–	–	x

Section A3 Physical and Chemical Properties of Active Substance

Annex point IIA, III 3

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.15 Explosive properties (IIA, III 3.11)	–	–	–	Not performed because the active substance does not present any risk for explosion.	–	–	–	x
3.16 Oxidizing properties (IIA, III 3.12)	–	–	–	Not performed because the active substance does not present oxidising properties.	–	–	–	x
3.17 Reactivity towards container material (IIA, III 3.13)	Not applicable (statement based on experience)	Specification: min. 96%	DCPP as manufactured is not packaged as such. Only the 30% formulation with the trade name [redacted] is placed on the market and imported by Ciba to Germany. Standard packaging item for [redacted] Polyethylene canister, 60 litre. Judged from the experience in use since many years [redacted] is not reactive towards the above mentioned container material.	–	N	2	[redacted]2007a and 2007b	x

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2011
Materials and methods	Acceptable with the amendments given below
Results and discussion	Agree with applicant's version with the amendments given below
Conclusion	Agree with applicant's version with the amendments given below
Reliability	Please see single subsections
Acceptability	Acceptable
Remarks	**3.1.1 Melting point:** Purity: > 99% GLP: Y Reliability: 1 Reference: This corresponds to Study A3.1/01. **3.1.2 Boiling point:** Purity: > 99% GLP: Y Reliability: 1 Reference: This corresponds to Study A3.1/02. **3.1.3 Relative density:** Purity: > 99% GLP: Y Reliability: 1 Results: The relative density is D^{20}_4=1.47. Reference: This corresponds to Study A 3.1/03. **3.1.3 Bulk density:** Purity: 99.97% GLP: N Reliability: 2 Results: Pour density = 0.45 g/mL; Tap density = 0.61 g/mL. Reference: This corresponds to Study A 3.1/04. **3.2 Vapour pressure:** Purity: > 99% GLP: N Reliability: 2 Results: The vapour pressure at 20°C is calculated according to the formula given in the study: It is $4.3*10^{-7}$ Pa. Reference: This corresponds to Study A3.2/01.

3.2.1 Henry's Law Constant:
Results: The estimation considered a temperature of 25°C.
Reliability: n.a.
Reference: This corresponds to Study A3.2/02.

3.3.1 Physical state:
Purity: 99.97%
Reliability: n.a. (visual inspection).
Reference: This corresponds to Study A3.3/01.

3.3.2 Colour:
Purity: 99.97%
Reliability: n.a. (visual inspection).
Reference: This corresponds to Study A3.3/01.

3.3.3 Odour:
Purity: 99.97%
Reliability: n.a.
Reference: This corresponds to Study A3.3/01.

3.4 Absorption spectra UV/VIS:
Purity: 99%
Remarks: There is an absorption maxima at 277 nm.
GLP: N
Reliability:2
Reference: This corresponds to Study A3.4/01.

3.4 Absorption spectra IR:
Purity: 99%
GLP: N
Reliability:2
Reference: This corresponds to Study A3.4/01.

3.4 Absorption spectra MS:
Purity: 99%
GLP: Y
Reliability:1
Reference: This corresponds to Study A3.3/01.

3.5 Solubility in water:

Method 1: Column elution method.

Purity: >99%

GLP: Y

Reliability:1

Results: Solubility at 20°C: 19.5 mg/L; pH 5-6.

Reference: This corresponds to Study A3.5/01.

Method 2: HPLC-UV

Purity: 99%

GLP: Y

Reliability:1

Results: pH 5 and 10°C 6.3 mg/L; pH 5 and 20°C 10 mg/L; pH 5 and 30°C 14.7mg/L.

Remarks: At higher pH values as the substance is on hand nearly undissociated.

pH 7 not measured. The pKa of DCPP is 9.49. Because DCPP has no further substituents beside the one hydroxyl group, which could be protonated, it is expected that the solubility at a lower pH than the pKa is in the range of 10 to 20 ppm as measured at pH 5-6 (i.e. solubility is rather independent from the actual pH as long as the pH is significantly below the pKa).

Reference: This corresponds to Study A3.5/02.

3.6 Dissociation constant

Purity: 99.97%

Reliability:1

GLP: Y

Results: The dissociation constant (pKa) was determined to be pKa=9.49 (20°C).

Reference: This corresponds to Study A3.6/01.

3.7 Solubility in organic solvents, including the effect of temperature on solubility

Purity: 99.97%

Reliability:1

GLP: N

Result: The study gives the results in g/kg; as the density of the testing solution is not given, a conversion into g/mL cannot be done; as a rough approximation, the density can be assumed to be 1, leading to the following values:

Solubility in n-hexane:
~ 8731 mg/L at 10 °C; ~ 18638 mg/L at 20 °C; ~ 27049 mg/L at 30 °C

Solubility in n-octanol:
~ 368228 mg/L at 10 °C; ~ 436764 mg/L at 20 °C; ~ 513828 mg/L at 30 °C

Reference: This corresponds to Study A 3.5/02.

3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products

Results: Company Statement; Not relevant. The active substance as manufactured does not include any organic solvent.

Reliability: n.a. (Company statement).

3.9 Partition coefficient n-octanol/water

Method 1: OECD Guideline 117

Purity: >99%

GLP: Y

Reliability: 1

Reference: This corresponds to Study A 3.9/01.

Method 2: Calculation.

Reliability: n.a.

Remarks: The log Pow determination as described in the study was carried out by experimental determination of the solubility of DCPP in water at pH 5 and in octanol. The subsequent calculation of the log Pow was based on these solubilities.

Reference: This corresponds to Study A 3.5/02.

3.10 Thermal stability, identity of relevant breakdown products

Purity/Specification: The purity is given by the company.

Method: DSC and TGA measurements.

Reliability:1

GLP: Y

Remarks: Based on DCS and TGA measurements, it can be concluded that the active substance is stable between 30 and 150°C.

Reference: This corresponds to Study A 3.10/01.

3.11 Flammability, including auto-flammability and identity of combustion products

Purity: 99.97%

GLP: Y

Reliability:1

Result: DCPP is not highly flammable.

Reference: This corresponds to Study A 3.11/01.

Auto-flammability:

Purity: 99.97%

Result: DCPP is not auto-flammable.

GLP: Y

Reliability:1

Reference: This corresponds to Study A 3.11/02.

3.12 Flash-point

Result: Not performed because the active substance is solid.

Reliability: n.a (Company Statement)

3.13 Surface tension

Purity: >99 %

GLP: Y

Reliability:1

Remarks: For the determination of the surface tension, 100.74 mg of the test

substance DCPP was diluted in a total volume of 100ml double distilled water. Prior to the measurement of the surface tension, 1 part of this solution was mixed with double distilled water.

Reference: This corresponds to Study A 3.13/01.

3.14 Viscosity

Result: Not performed because the active substance is solid.

Reliability: n.a (Company Statement)

3.15 Explosive properties

Remarks/Justification: Furthermore there is no structural alert for explosive properties.

Remarks: n.a (Company Statement).

3.16 Oxidizing properties

Remarks/Justification: Furthermore there is no structural alert for oxidizing properties.

Remarks: n.a (Company Statement).

3.17 Reactivity towards container material

Results: Standard packaging item for [redacted] polyethylene canister, 60 Liter. Judged from the experience in use since many years [redacted] is not reactive towards the above mentioned container material.

Reference: This corresponds to Study A 3.17/01 and to Study A 3.17/02.

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1 Melting point, boiling point, relative density (IIA, III 3.1)								
3.1.1 Melting point	–	–	No data available	The substance is a degradation metabolite which does not manufacture and market therefore the study does not need to be performed.	–	–	–	
3.1.2 Boiling point	–	–	No data available	The substance is a degradation metabolite which does not manufacture and market therefore the study does not need to be performed.	–	–	–	
	Calculation based on Adapted Stein and Brown Method using MPBPVP (v1.43) module of software EPI Suite v.4.11	–	343.7°C	–	N	2	EPI Suite v4.11, online query 29.05.2013	x
	Calculation using ACD/Labs software v11.02 cited in SciFinder.	–	347.1°C at 760 Torr	–	N	2	SciFinder, online query 05.08.2013	x

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.1.3 Bulk density/ relative density								
Density	Calculation using ACD/Labs software v11.02 cited in SciFinder.	–	1.294 kg/m^3 at 20°C	–	N	2	SciFinder, online query 05.08.2013	x
3.2 Vapour pressure and Henry´s Law Constant (IIA, III 3.2)								
Vapour pressure	Calculation based on Modified Grain Method using MPBPVP (v1.43) module of software EPI Suite v.4.11 as recommended in REACH guideline	–	3.58E-3 Pa at 25 °C	–	N	2	EPI Suite v4.11, online query 29.05.2013	x
	Calculation using ACD/Labs software v11.02 cited in SciFinder.	–	1.10E-4 Torr at 25°C (=1.47E-2 Pa)	–	N	2	SciFinder, online query 05.08.2013	x

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
Henry's Law Constant	Calculation based on QSAR method using HENRYWIN (v3.20) module of software EPI Suite v4.11 as recommended in REACH guideline.	–	Results at 25 °C: 0.388 Pa.m^3/mol (Bond method) 16.8 Pa.m^3/mol (Group method)	–	N	2	EPI Suite v4.11, online query 29.05.2013	x
3.3 Appearance (IIA, III 3.3)								
3.3.1 Physical state	Visual inspection	–	Powder	–	–	–	–	x
3.3.2 Colour	Visual inspection	–	White	–	–	–	–	x
3.3.3 Odour	–	–	–	–	–	–	–	
3.4 Absorption spectra (IIA, III 3.4)								
UV/VIS	–	–	–	–	–	–	–	

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
IR	In accordance with internal standard operation procedure The IR spectrum was recorded on a FTS 375C FT-IR spectrometer. The sample was prepared as KBr pellet.	[redacted] Batch No.: [redacted]A Purity: 99.7% by GC and HPLC	Methyl-DCPP was identified by FT-IR spectrum. All bands observed are well in accordance with the proposed structure. .	–	N	1	[redacted] 02.09.2002	x
NMR	In accordance with internal standard operation procedure. NMR spectra were acquired on an ^{UNITY}INOVA 500 NMR spectrometer. The experiment was performed at 25°C.	[redacted] Batch No.: [redacted] Purity: 99.7% by GC and HPLC	The ^{1}H-NMR spectra were measured in $CDCI_3$ and DMSO-d_6 The structure was confirmed by NMR measurements	–	N	1	[redacted] 02.09.2002	x

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
MS	In accordance with internal standard operation procedure The El-MS spectrum was acquired on a MAT 95 mass spectrometer. The sample was introduced via direct inlet probe.	[redacted] Batch No.: [redacted] Purity: 99.7% by GC and HPLC	The structure proposed can be assigned to the El mass spectrum of the sample.	–	N	1	[redacted] 02.09.2002	x
3.5 Solubility in water (IIA, III 3.5)	OECD guideline 105 (column elution method)	[redacted] Batch No.: [redacted] Purity: 99.7%	0.322 mg/L at 20 °C at pH 6.95	–	Y	1	[redacted] 10.03.2003	x
3.6 Dissociation constant (-)	–	–	Not applicable	The substance does not contain any ionisable functional groups therefore the study does not need to be performed	–	–	–	

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.7 Solubility in organic solvents, including the effect of temperature on solubility (IIIA, III 1)	–	–	–	–	–	–	–	
3.8 Stability in organic solvents used in b.p. and identity of relevant breakdown products (IIIA, III 2)	–	–	–	–	–	–	–	
3.9 Partition coefficient n-octanol/water (IIA, III 3.6)	Calculation based on fragment method using KOWWIN (v1.68) module of software EPI Suite v4.11 as recommended in REACH guideline.	–	Log Kow= 4.58 at 25°C	–	N	2	EPI Suite v4.11, online query 29.05.2013	x
	Calculation using ACD/Labs software v11.02 cited in SciFinder.	–	Log P= 4.84 at 25°C	–	N	2	SciFinder, online query 05.08.2013	x
3.10 Thermal stability, identity of relevant breakdown products (IIA, III 3.7)	–	–	–	–	–	–	–	

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.11 Flammability, including auto-flammability and identity of combustion products (IIA, III 3.8)								
Flammability	–	–	- No data available on flammability, A.10 - The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.	- The substance is a degradation metabolite which does not manufacture and market therefore the study on flammability A.10 does not need to be performed. - Based on chemical structure and experience in handling and use of substance the pyrophoric properties and the flammability in contact with water are not to be expected.	–	–	–	x
Auto-flammability	–	–	No data available	The substance is a degradation metabolite which does not manufacture and market therefore the study does not need to be performed	–	–	–	x

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.12 Flash-point (IIA, III 3.9)	–	–	Not applicable	The substance is a solid therefore the study does not need to be performed.	–	–	–	x
3.13 Surface tension (IIA, III 3.10)	–	–	Not surface-acitve	Based on structure, surface activity is not expected and the water solubility is below 1mg/L at 20°C respectively therefore the study does not need to be performed.	–	–	–	x
3.14 Viscosity (-)	–	–	Not applicable	The substance is a solid therefore the study does not need to be performed.	–	–	–	x
3.15 Explosive properties (IIA, III 3.11)	–	–	No explosive properties	There are no chemical groups associated with explosive properties present in the molecule therefore the study does not need to be performed.	–	–	–	x

Section A3 Physical and Chemical Properties of Methyl-DCPP / CAS 4640-07-7

Subsection (Annex Point)	Method	Purity/ Specification	Results	Remarks/ Justification	GLP (Y/N)	Reliability	Reference	Official use only
3.16 Oxidizing properties (IIA, III 3.12)	–	–	No oxidising properties	On the basis of the chemical structure the substance is incapable of reacting exothermically with combustible materials therefore the study does not need to be performed.	–	–	–	x
3.17 Reactivity towards container material (IIA, III 3.13)	–	–	–	–	–	–	–	

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2013
Materials and methods	Acceptable with the amendments given below
Results and discussion	Agree with applicant's version with the amendments given below
Conclusion	Agree with applicant's version with the amendments given below
Reliability	Please see single subsections
Acceptability	Acceptable
Remarks	**3.1.1 Melting point:** Remarks/Justification: The substance is a degradation metabolite which does not manufacture and market therefore the study does not need to be performed. **3.1.2 Boiling point:** Calculation 1 Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.1.02.EPISuite, M-DCPP Calculation 2 Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.1.02.SciFinder, M-DCPP **3.1.3 Relative density:** Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.1.03.SciFinder, M-DCPP **3.2 Vapour pressure:** Calculation 1 Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.2/01.EPISuite, M-DCPP Calculation 2 Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.2/02.SciFinder, M-DCPP **3.2.1 Henry's Law Constant:** Reliability: n.a.(Calculation) Reference: This corresponds to Study A3.2/01.EPISuite, M-DCPP **3.3.1 Physical state:** Purity: 99.7% Reliability: n.a. (visual inspection). Reference: This corresponds to Study A3.5. M-DCPP **3.3.2 Colour:** Purity: 99.7% Reliability: n.a. (visual inspection).

Reference: This corresponds to Study A3.5. M-DCPP

3.4 Absorption spectra IR:

Purity: 99.7%

Batch: E[redacted]

GLP: N

Reliability:2

Reference: This corresponds to Study A3.4.M-DCPP

3.4 Absorption spectra NMR:

Purity: 99.7%

Batch[redacted]

GLP: N

Reliability:2

Reference: This corresponds to Study A3.4.M-DCPP

3.4 Absorption spectra MS:

Purity: 99.7%

Batch:[redacted]

GLP: N

Reliability:2

Reference: This corresponds to Study A3.4.M-DCPP

3.5 Solubility in water:

Purity: 99.7%

Batch: E[redacted]

GLP: Y

Reliability:1

Reference: This corresponds to Study A3.5.M-DCPP

3.6 Dissociation constant

Remarks/Justification: The substance does not contain any ionisable functional groups therefore the study does not need to be performed

3.9 Partition coefficient n-octanol/water

Calculation 1

Reliability: n.a.(Calculation)

Result:log Pow 4.58 at 25°C

Reference: This corresponds to Study A3.9.EPISuite, M-DCPP

Calculation 2

Reliability: n.a.(Calculation)

Result:log Pow 4.84 at 25°C

Reference: This corresponds to Study A3.9.SciFinder, M-DCPP

3.11 Flammability, including auto-flammability and identity of combustion products

Results: The substance has no pyrophoric properties and does not liberate

flammable gases on contact with water.

Remarks/Justification: The substance is a degradation metabolite which does not manufacture and market therefore the study on flammability A.10 does not need to be performed.

- Based on chemical structure and experience in handling and use of substance the pyrophoric properties and the flammability in contact with water are not to be expected.

Auto-flammability:

Remarks/Justification: The substance is a degradation metabolite which does not manufacture and market therefore the study does not need to be performed.

3.12 Flash-point

Remarks/Justification: The substance is a solid therefore the study does not need to be performed.

3.13 Surface tension

Result: not surface-active

Remarks/Justification: Based on structure, surface activity is not expected and the water solubility is below 1mg/L at 20°C respectively therefore the study does not need to be performed

3.14 Viscosity

Remarks/Justification: The substance is a solid therefore the study does not need to be performed.

3.15 Explosive properties

Result: No explosive properties

Remarks/Justification: There are no chemical groups associated with explosive properties present in the molecule therefore the study does not need to be performed.

3.16 Oxidizing properties

Result: No oxidizing properties

Remarks/Justification: On the basis of the chemical structure the substance is incapable of reacting exothermically with combustible materials therefore the study does not need to be performed.

Section A4.2a Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Official use only

1 REFERENCE

1.1 Reference

1) [redacted] 2008a, Determination of DCPP in water and soil samples with LC/MS/MS. [redacted] Document / SOP No. A.TA.106.01 (unpublished), Date: 2008-03-28

2) [redacted] 2008b, Method check / validation of the method A.TA.106 for soil samples. [redacted] Report No. 07.496B (unpublished), Date: 2008-03-28

1.2 Data protection

Yes

1.2.1 Data owner

BASF SE

1.2.2 Companies with letter of access

–

1.2.3 Criteria for data protection

Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No,
no guideline available

2.2 GLP

No

2.3 Deviations

No

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

3.1.2 Cleanup

No enrichment or clean-up is necessary. 2 g of soil are spiked with $^{13}C_6$-triclosan as internal standard. The sample is extracted three times with 5 mL acetone/hexane 1:1 (v/v) each in an ultrasound-bath (15 min). After centrifugation the extract is transferred to a 20 mL volumetric flask and the combined extracts are filled up to volume with acetone/hexane 1:1 (v/v). The extract is dried with Na-sulphate and an aliquote of the dried extract is filtered with a 0.45 µm PTFE filter. 1 mL of this extract is concentrated to dryness with N_2 and reconstituted with 1 mL acetonitrile. The final extract is analysed according to the indicated conditions.

3.2 Detection

3.2.1 Separation method

Chromatographic conditions:

Column:	e.g.Phenomenex Synergy Max RP 75 x 2 mm; 4 µm particle
Column temp.:	Room temperature
Flow:	300 µL/min
Eluent A:	Water with 0.1% formic acid
Eluent B:	Methanol
Gradient elution	
Injection:	e.g. 20 µL (depending on concentration range)

Section A4.2a Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

3.2.2	Detector	Mass spectrometric detector.
3.2.3	Standard(s)	Quantification is done using internal standard ($^{13}C_6$-triclosan).
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.
3.3	**Linearity**	
3.3.1	Calibration range	Six concentrations in the range of 5 µg/kg to 500 µg/kg were analysed.
3.3.2	Number of measurements	Each concentration was measured once.
3.3.3	Linearity	Correlation coefficient: 0.999
3.4	**Specificity: interfering substances**	No interferences were observed.
3.5	**Recovery rates at different levels**	Two soil samples were each fortified with 20 µg/kg and 500 µg/kg of active substance. Each fortified sample was analysed three times. The found values were compared with the nominal spiked values. The recoveries for the low concentration level ranged from 105.5% to 119.5% and for the high concentration level from 91.4% to 99.6%. The mean recovery was 112.9% (n=6) for the low concentration level and 95.8% (n=6) for the high concentration level.
3.5.1	Relative standard deviation	The relative standard deviation for the low concentration level was 5.4% (n=6) and for the high concentration level 3.2% (n=6).
3.6	**Limit of determination**	1 µg/kg
3.7	**Precision**	
3.7.1	Repeatability	Two soil samples were each fortified with 20 µg/kg and 500 µg/kg of active substance. Each fortified sample was analysed three times. The relative standard deviation for the low concentration level was 5.4% (n=6) and for the high concentration level 3.2% (n=6).
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of the active substance in soil samples are analysed by means of liquid chromatography using mass spectrometric detection after extraction with acetone/hexane 1:1. Quantification is done using internal standard.
4.2	**Conclusion**	The analytical method was sufficiently validated with respect to specificity, linearity, precision and accuracy. The method is therefore suitable for the determination of DCPP residues in soil.
4.2.1	Reliability	Reliability indicator: 2
4.2.2	Deficiencies	No

Section A4.2a Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2

ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN SOIL

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2009
Materials and methods	Acceptable
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	See also section A4.2a_01

Section A4.2b Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 2001, Ambient monitoring method for triclosan [redacted] in air. [redacted] Document No.: A.OH.145.01 (unpublished), Date: 2001-02-28 The method was developed in the past for the analysis of triclosan but could also be used for the analysis of DCPP. (Reference: Hauk, 2007c)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No No guideline available
2.2	**GLP**	No
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Sampling is performed by collecting the active substance on glass fiber filters using a calibrated pump. The sampling volume is 60-480 L air. The sampling speed is 2 L/min and the sampling time 30-240 min. After sampling the filter is transferred to a 4 mL flask. 2 mL of extraction solution (ethyl acetate) are added. Extraction is accomplished within 15 minutes using an ultrasonic bath. Before analysis, the solutions are filtered through a Gelman filter.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Chromatographic conditions: Column: Fused silica, 15 m, ID = 0.52 mm, stationary phase: DB-5, 1.0 µm Carrier gas: helium, 51 cm/s Temperatures: Column: 2 min at 60 °C → 40 °C/min → 215 °C → 10 °C/min → 10 min at 250 °C Injector: on column, 30 s at 60 °C → 150 °C/min → 22 min at 215 °C Injection volume: 1.0 µL, on column Retention time: 10.06 min
3.2.2	Detector	Flame ionisation detector (FID), range = 12, attenuation 8

Section A4.2b Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

3.2.3	Standard(s)	External standard
3.2.4	Interfering substance(s)	Substances of sample matrix or adsorption material may interfere with the active substance.
3.3	**Linearity**	
3.3.1	Calibration range	Calibrated range: 6 to 120 µg/filter corresponding to 0.1 to 2 mg/m^3 for 60 L air and 120 to 1200 µg/filter corresponding to 0.25 to 2.5 mg/m^3 for 480 L air.
3.3.2	Number of measurements	Each concentration was measured once.
3.3.3	Linearity	Correlation coefficient: 0.9965
3.4	**Specificity: interfering substances**	No interferences were observed.
3.5	**Recovery rates at different levels**	Recovery determination was made by spiking glass fiber filters with a known quantity of the active substance at five concentration levels: 6.20, 59.85, 124.40, 478.0 and 1197.0 µg. Each level was measured five times. The mean recoveries ranged from 93.25% to 95.78%.
3.5.1	Relative standard deviation	The relative standard deviations ranged from 1.31% to 9.68%. The overall relative standard deviation for the overall sampling and analytical part was 5.43%.
3.6	**Limit of determination**	0.1 µg/filter corresponding to 2 µg/m^3 air (60 L air).
3.7	**Precision**	
3.7.1	Repeatability	Please refer to point 3.5 (recovery rates).
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Sampling is performed by collecting the active substance on glass fiber filters using a calibrated pump. Following extraction with ethyl acetate, the sample solutions are analysed by gas chromatography using flame ionisation detection.
4.2	**Conclusion**	The analytical method has been validated successfully by checking the parameters linearity, specificity, accuracy, precision as well as the limit of determination. The method is therefore suitable for the determination of active substance residues in air samples.
4.2.1	Reliability	Reliability indicator: 2
4.2.2	Deficiencies	No

Section A4.2b — Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 — ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN AIR

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2014
Materials and methods	The vapour pressure of DCPP is 1.2*10-06 Pa at 25 °C which is clearly below the trigger value. Since no spray application is foreseen within the intended uses described in this CAR, so no method for air is necessary. The study submitted for the analysis of DCPP in air describes a method developed for Triclosan. No validation data for DCPP has been submitted for this method so far. Although a similar behaviour of DCPP can be expected, a separate validation study is necessary when relevant at product authorisation stage.
Conclusion	Not applicable.
Reliability	Not applicable.
Acceptability	Not applicable.
Remarks	-

Section A4.2c Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Official use only

1 REFERENCE

1.1 Reference

1) [redacted], 2008a, Determination of DCPP in water and soil samples with LC/MS/MS. [redacted] Document / SOP No. A.TA.106.01 (unpublished), Date: 2008-03-28

2) [redacted]R., 2008c, Method check / validation of the method A.TA.106 for water samples. [redacted] Report No. 07.496A (unpublished), Date: 2008-03-26

1.2 Data protection Yes

1.2.1 Data owner: BASF SE

1.2.2 Companies with letter of access: –

1.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No,
no guideline available

2.2 GLP No

2.3 Deviations No

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment
3.1.2 Cleanup

The well mixed sample is diluted 1:1 with methanol and spiked with $^{13}C_6$-triclosan as internal standard. The sample is treated in an ultrasonic bath (1-2 min) and centrifuged. The extract is then analysed according to the indicated conditions. If a lower limit of quantification is required, sample enrichment with solid phase extraction (SPE) can be performed.

3.2 Detection

3.2.1 Separation method

Chromatographic conditions:

Column:	e.g.Phenomenex Synergy Max RP 75 x 2 mm; 4 µm particle
Column temp.:	Room temperature
Flow:	300 µL/min
Eluent A:	Water with 0.1% formic acid
Eluent B:	Methanol
Gradient elution	
Injection:	e.g. 20 µL (depending on concentration range)

3.2.2 Detector: Mass spectrometric detector.

3.2.3 Standard(s): Quantification is done using internal standard ($^{13}C_6$-triclosan).

3.2.4 Interfering substance(s): Substances of specimen matrix may interfere.

Section A4.2c — Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 — ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

3.3	**Linearity**	
3.3.1	Calibration range	Six concentrations in the range of 0.1 µg/L to 50 µg/L were analysed.
3.3.2	Number of measurements	Each concentration was measured once.
3.3.3	Linearity	Correlation coefficient: 0.999
3.4	**Specificity: interfering substances**	No interferences were observed.
3.5	**Recovery rates at different levels**	10 µg/L of active substance were spiked in drinking water and waste water. Both samples were analysed three times. Additional drinking water was spiked with 1 µg/L DCPP and analysed three times. The found values were compared with the nominal spiked values. The recoveries found for drinking water spiked with 1 µg/L ranged from 95.2% to 98.3% and recoveries found for drinking water spiked with 10 µg/L ranged from 98.5% to 100.0%. The recoveries found for waste water were between 98.6% and 103.0%. The overall mean recovery was 99.3%.
3.5.1	Relative standard deviation	The overall relative standard deviation was 2.5%.
3.6	**Limit of determination**	0.1 µg/L
3.7	**Precision**	
3.7.1	Repeatability	10 µg/L of active substance were spiked in drinking water and waste water. Both samples were analysed three times. The relative standard deviation of all six determinations was 2.0%. Additional drinking water was spiked with 1 µg/L DCPP and analysed three times to check the precision for lower concentrations. The relative standard deviation was 1.6%.
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of the active substance in water samples are analysed by means of liquid chromatography using mass spectrometric detection directly after addition of methanol. Quantification is done using internal standard.
4.2	**Conclusion**	The analytical method was sufficiently validated with respect to specificity, linearity, precision and accuracy. The method is therefore suitable for the determination of DCPP residues in water.
4.2.1	Reliability	Reliability indicator: 2
4.2.2	Deficiencies	No

Section A4.2c — Analytical Methods for Detection and Identification

Annex Point IIA, IV 4.2 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN WATER

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2009
Materials and methods	Acceptable
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	-

Section A4.2 **Annex Point IIA, IV 4.2**	**Analytical Methods for Detection and Identification** ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN ANIMAL AND HUMAN BODY FLUIDS AND TISSUES	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Since the active substance DCPP is not classified as toxic or highly toxic it is justified not to submit an analytical method for its determination in animal and human body fluids and tissues.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2009	
Evaluation of applicant's justification	Acceptable	
Conclusion	Agree with applicant’s version	
Remarks	-	

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

Official use only

1 REFERENCE

1.1 Reference

[redacted]2000, Determination of [redacted]Diclosan) in Samples of a Biodegradation Study. [redacted] Document / PI phase report to GLP study G594 13 from Solvias (unpublished), Date: 2001-01-12

1.2 Data protection Yes

1.2.1 Data owner: BASF SE

1.2.2 Companies with letter of access: Not applicable, since data submitter is data owner

1.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No data

2.2 GLP Yes

2.3 Deviations Not applicable

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

3.1.2 Cleanup

The sludge samples were extracted and worked up analogous to the water sample, with the exception, that the sludge particles were filtered during the transfer of the sample to the thimble. Therefore an filter device was connected with the thimble between the funnel and the inlet side of the thimble. The sludge suspension was pipetted into the funnel and sucked through the filter and the thimble packing. After the sampling the filter was removed from the filter device and inserted into the thimble (at the inlet side).

Enrichment with SFE timble:

SFE thimble (7 ml volume) packed with glass wool and Tenax as adsorbent.

The sludge suspension is sucked through the thimble packing (with filter device. At the end of sampling some air is sucked trough the thimble packing and the timble is freeze dried in a lyophilisator. Thereafter the thimble is extracted with SFE.

The extraction of the thimble is carried out with a SFE apparatus under the following operating conditions:

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

The final hexane extract *is* evaporated under N_2 to a volume of about 0.5 ml and approx. 0.5 ml derivatising reagent N,N-diethyl-trimethylsilylamine *is* added.

HP 7680T SFE module, controlled by the SFE ChemStation software version B.01.02

extraction medium	pure SFE grade CO_2
extraction chamber temperature	80°C
static modifier	60 µl formic acid
extraction steps	379 bar, 15 min static followed by 25 min dynamic
CO_2 flow rate (dynamic step)	1 ml/min
nozzle temperature	45 °C
trap (material)	octadecyl-silica (ODS trap purchased by Hewlett-Packard)
trap temperature (sorption step)	10 °C
trap rinsing solvent	1.5 ml hexane (p.A)
trap volume compensation	1.0 ml ± 0.1 ml
trap rinsing flow	1 ml/min
trap temperature (rinse step)	20°C

3.2 Detection

3.2.1 Separation method

GC/MSD (HP 5890 GC coupled with a HP 5970 mass selective detector)
1 µl of the extract is injected via autosampler in a split/splitless injector at 250°C in the splitless mode (1 min). A DB5-type column (30 m, 0.25 mm i.d., 0.25 µm film) and helium as carrier gas is used. The column temperature is held constant at 60°C for 1 min after injection, then increased to 180°C at a rate of 5°C/min, followed by an temperature increase of 15°C/min to 280°C (held at 280°C for at least 10 min). The transfer line temperature to MSD is const. 260°C.

3.2.2 Detector

Mass spectrometric detector.

Data acquisition is done in SIM mode (target ions used for quantification areprinted in bold)

Target substances	RT (approx. min)	Ions (m/z)
Me-O-DCS	29.8	268, **270**
Si-13C-TCS	31.2	351, **353**

3.2.3 Standard(s)

Quantification is done using internal standard (13C6-triclosan).

3.2.4 Interfering substance(s)

Substances of specimen matrix may interfere.

3.3 Linearity

3.3.1 Calibration range

Four concentrations in the range of 40 ng/ml to 400 ng/mL extract were analysed.
Quantification is carried out via the isotope dilution method with individual response factors for each compound according to the following equation
Calculation of individual response factors for the component (i)

Section A4.2 Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

Annex Point IIA, IV.4.2

		from the reference injection: $$x_i = \frac{\left[c_i^{Rf}\right]}{\left[c_{Std}^{Rf}\right]} \cdot \frac{Int_{Std}^{Rf}}{Int_i^{Rf}}$$	
3.3.2	Number of measurements	*Single analysis for each concentration level*	
3.3.3	Linearity	Correlation coefficient: 1.000	
3.4	**Specifity: interfering substances**	*none*	
3.5	**Recovery rates at different levels**	*64% ± 15% (over all analyzed samples)*	
3.5.1	Relative standard deviation	*Data not available*	
3.6	**Limit of determination**	*Limit of detection: 5 ng absolute (5 ng/ml extract)*	X
3.7	**Precision**		
3.7.1	Repeatability	*Data not available*	
3.7.2	Independent laboratory validation	*Data not available*	
		4 APPLICANT'S SUMMARY AND CONCLUSION	
4.1	**Materials and methods**	Enrichment with SFE timble. There after the thimble is extracted with SFE. Analysis of the extract with GC/MSD (HP 5890 GC coupled with a HP 5970 mass selective detector) and internal calibration. Quantification is carried out via the isotope dilution method with individual response factors for each compound.	
4.2	**Conclusion**	The validation results confirm that the method is suitable for the determination of Me-O-DCPP in sewage sludge samples.	
4.2.1	Reliability	2	
4.2.2	Deficiencies	No	

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2013*
Materials and methods	Section 3.6:only the detection limit for the extract solution is given. Since there no sample amounts corresponding to the given extract volumes are given it is not possible to calculate detection limit as ng per g sample
Conclusion	*Agree with applicants version with the amendments given above*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	-

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] 2001, Determination of o-, m-, p-Chloroanisols, o-, m-, p-Chlorophenol, Methoxy-Diclosan and Diclosan in Samples of the Biodegradation Study for [redacted] Document / PI phase report to GLP study G641 13 from Solvias (unpublished), Date: 2001
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable, since data submitter is data owner
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No data
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Prior to analysis, 10 µl internal standard solution (Methoxy-Triclosan 150 ng/µl) were spiked to the sludge and 3 ml water were added. Thereafter the sludge pellet were re-suspended under ultrasonic treatment and transferred to a 6 ml headspace-vial. 8 pellets NaOH and 50 µl diethylsulfate (DES) were added and the sample heated for 1 h by 80°C. After the sample was cooled to room temperature 200 µl Acetonitrile were added and heated once again by 80°C for ½ h. After cooling to room temperature 1 ml extractand (1/5 toluene 4/5 hexane) and 10 µl injection standard solution (PCB 28 100 ng/µl) were added, well shaked for at least 1 min and the organic layer analysed by GC/MS
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	The analysis of the DES-derivatised extracts was carried out with a GC/MS (Finnigan GCQ Iontrap-MS system) without further clean up. 2 µl of the extracts were injected via autosampler in a split/split less injector at 250°C in the split less mode (1 min). A MN Optima delta 6 column (30 m, 0.25 mm ID, 0.25 µm film) and helium as carrier gas was used. The column temperature was held constant at 50°C for 1 min after injection, then increased to 280°C at a rate of 10°C/min (held at 280°C for 10 min). The transfer line temperature to MS was const. 250°C, the source temp 180°C.
3.2.2	Detector	Iontrap-mass spectrometric detector.

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

		Data acquisition is done in fullscan mode (selected ions used for quantification are printed in bold)	

Target substances	RT (approx. min)	Ions (m/z)
Me-O-DCS	26.0	**268**, 270
Methoxy-Triclosan	27.3	**302**, 304

3.2.3	Standard(s)	Quantification is done using internal standard (Methoxy-Triclosan).	
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.	
3.3	**Linearity**		
3.3.1	Calibration range	Five concentrations in the range of 50-5000 ng/ml extract were analysed.	
3.3.2	Number of measurements	*concentration level analyzed in triplicate*	
3.3.3	Linearity	Correlation coefficient: 0.997	
3.4	**Specifity: interfering substances**	*none*	
3.5	**Recovery rates at different levels**	*86% (over all analyzed samples)*	
3.5.1	Relative standard deviation	*15.2%*	
3.6	**Limit of determination**	*Limit of detection: 25 ng/ml extract*	X
3.7	**Precision**		
3.7.1	Repeatability	*5.4% at 250 ng/ml extract (n=3)*	
3.7.2	Independent laboratory validation	*Data not available*	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	GC/MS (Finnigan GCQ Iontrap-MS system) by means of internal calibration without further clean up. Quantification was carried out via the calibration curve equation of a multi-point-calibration.
4.2	**Conclusion**	The validation results confirm that the method is suitable for the determination of Me-O-DCPP in sewage sludge samples.
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Soil

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2013*
Materials and methods	Section 3.6:only the detection limit for the extract solution is given. Since there no sample amounts corresponding to the given extract volumes are given it is not possible to calculate detection limit as ng per g sample
Conclusion	*Agree with applicants version with the amendments given above*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	-

Section A4.2 Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

Annex Point IIA, IV.4.2

Official use only

1 REFERENCE

1.1 Reference

[redacted]2001, Determination of o-, m-, p-Chloroanisols, o-, m-, p-Chlorophenol, Methoxy-Diclosan and Diclosan in Samples of the Biodegradation Study for [redacted]
Document / PI phase report to GLP study G641 13 from Solvias (unpublished), Date: 2001

1.2 Data protection

Yes

1.2.1 Data owner

BASF SE

1.2.2 Companies with letter of access

Not applicable since data submitter is data owner

1.2.3 Criteria for data protection

Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No data

2.2 GLP

Yes

2.3 Deviations

Not applicable

3 MATERIALS AND METHODS

3.1 Preliminary treatment

3.1.1 Enrichment

3.1.2 Cleanup

Prior to analysis, the samples were acidified with phosphoric acid to reach a pH value of approx. 2. 10 ml of the sample were spiked with 10 µl internal standard solution (Methoxy-Triclosan 150 ng/µl)
The prepared water sample were extracted with SPE under following conditions:

SPE-cartridge:	*Abselute Nexus 12cc/200mg*
conditioning:	*none*
sample addition:	*slowly add the prepared sample*
washing:	*1 x 5 ml water pH 2 (with formic acid)*
	dry with air for 2 minutes
elution:	*2 x 1,5 ml diethyl ether*

100 µl toluene were added as keeper and the extract evaporated slowly under N2 to 1 ml. 10 µl injection standard solution (PCB 28 100 ng/µl) were added to the evaporated extract. To 100 µl of this extract 100 µl derivatising solution were added and analysed by GC/MSD

derivatising solution:	*2/3 HMDS*
	1/3 Toluene

3.2 Detection

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

3.2.1	Separation method	The analysis of the HMDS-derivatised extracts was carried out with a GC/MSD (HP 5890 GC coupled with a HP 5971 mass selective detector) without further clean up. 1 µl of the extracts was injected via autosampler in a split/splitless injector at 250oC in the splitless mode (1 min). A DB5-type column (Supelco PTE 5; 30 m, 0.25 mm ID, 0.25 µm film) and helium as carrier gas was used. The column temperature was held constant at 40°C for 1 min after injection, then increased to 12°C at a rate of °C/min, followed by an temperature increase of 1°C/min to 23°C and then 6°C/min to 280°C (hold at 28°C for 2.33 min). The transfer line temperature to MSD was const. 260°C.
3.2.2	Detector	Mass spectrometric detector. Data acquisition is done in SIM mode (target ions used for quantification are printed in bold)

Target substances	RT (approx. min)	Ions (m/z)
Me-O-DCS	26.0	218, **268**, 270
Methoxy-Triclosan	27.3	**302**, 304

3.2.3	Standard(s)	Quantification is done using internal standard (Methoxy-Triclosan).
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.
3.3	**Linearity**	
3.3.1	Calibration range	Five concentrations in the range of 2-200 µg/l were analysed.
3.3.2	Number of measurements	*Single analysis for each concentration level*
3.3.3	Linearity	Correlation coefficient: 0.999
3.4	**Specifity: interfering substances**	*none*
3.5	**Recovery rates at different levels**	*94% (over all analyzed samples)*
3.5.1	Relative standard deviation	*8.7%*
3.6	**Limit of determination**	*Limit of detection: 2 µg/L*
3.7	**Precision**	
3.7.1	Repeatability	*13.1% at 10 µg/l (n=4)*
3.7.2	Independent laboratory validation	*Data not available*

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	GC/MSD (HP 5890 GC coupled with a HP 5971 mass selective detector) by means of internal calibration without further clean up. Quantification was carried out via the calibration curve equation of a multi-point-calibration.
4.2	**Conclusion**	The validation results confirm that the method is suitable for the determination of Me-O-DCPP in water samples.
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2013*
Materials and methods	*Acceptable*
Conclusion	*Agree with applicants version*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	-

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]2000, Determination of [redacted](Diclosan) in Samples of a Biodegradation Study. [redacted] Document / PI phase report to GLP study G594 13 from Solvias (unpublished), Date: 2001-01-12
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since data submitter is data owner
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No data
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	Sample is acidified with phosphoric acid to pH <2 and spiked with $^{13}C_6$-triclosan as internal standard. Enrichment with SFE timble: SFE thimble (7 ml volume) packed with glass wool and Tenax as adsorbent. The water sample (e.g. 60 ml) is sucked through the thimble packing. At the end of sampling some air is sucked trough the thimble packing and the timble is freeze dried in a lyophilisator. Thereafter the thimble is extracted with SFE. The extraction of the thimble is carried out with a SFE apparatus under the following operating conditions:
3.1.2	Cleanup	

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

HP 7680T SFE module, controlled by the SFE ChemStation software version B.01.02

extraction medium	pure SFE grade CO_2
extraction chamber temperature	80°C
static modifier	60 µl formic acid
extraction steps	379 bar, 15 min static followed by 25 min dynamic
CO_2 flow rate (dynamic step)	1 ml/min
nozzle temperature	45 °C
trap (material)	octadecyl-silica (ODS trap purchased by Hewlett-Packard)
trap temperature (sorption step)	10 °C
trap rinsing solvent	1.5 ml hexane (p.A)
trap volume compensation	1.0 ml ± 0.1 ml
trap rinsing flow	1 ml/min
trap temperature (rinse step)	20°C

The final hexane extract *is* evaporated under N_2 to a volume of about 0.5 ml and approx. 0.5 ml derivatising reagent N,N-diethyl-trimethylsilylamine *is* added.

3.2 Detection

3.2.1 Separation method

GC/MSD (HP 5890 GC coupled with a HP 5970 mass selective detector)
1 µl of the extract is injected via autosampler in a split/splitless injector at 250°C in the splitless mode (1 min). A DB5-type column (30 m, 0.25 mm i.d., 0.25 µm film) and helium as carrier gas is used. The column temperature is held constant at 60°C for 1 min after injection, then increased to 180°C at a rate of 5°C/min, followed by an temperature increase of 15°C/min to 280°C (held at 280°C for at least 10 min). The transfer line temperature to MSD is const. 260°C.

3.2.2 Detector

Mass spectrometric detector.

Data acquisition is done in SIM mode (target ions used for quantification areprinted in bold)

Target substances	RT (approx. min)	Ions (m/z)
Me-O-DCS	29.8	268, **270**
Si-13C-TCS	31.2	351, **353**

3.2.3 Standard(s)

Quantification is done using internal standard (13C6-triclosan).

3.2.4 Interfering substance(s)

Substances of specimen matrix may interfere.

3.3 Linearity

3.3.1 Calibration range

Four concentrations in the range of 40 ng/ml to 400 ng/mL extract were analysed.
Quantification is carried out via the isotope dilution method with individual response factors for each compound according to the following equation
Calculation of individual response factors for the component (i) from the reference injection:

Section A4.2 Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

Annex Point IIA, IV.4.2

$$x_i = \frac{\left[c_i^{Rf}\right]}{\left[c_{Std}^{Rf}\right]} \cdot \frac{Int_{Std}^{Rf}}{Int_i^{Rf}}$$

3.3.2	Number of measurements	*Single analysis for each concentration level*	
3.3.3	Linearity	Correlation coefficient: 1.000	
3.4	**Specifity: interfering substances**	*none*	
3.5	**Recovery rates at different levels**	*82% ± 20% (over all analyzed samples)*	
3.5.1	Relative standard deviation	*Data not available*	
3.6	**Limit of determination**	*Limit of detection: 5 ng absolute (5 ng/ml extract)*	X
3.7	**Precision**		
3.7.1	Repeatability	*Data not available*	
3.7.2	Independent laboratory validation	*Data not available*	

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Enrichment with SFE timble. There after the thimble is extracted with SFE. Analysis of the extract with GC/MSD (HP 5890 GC coupled with a HP 5970 mass selective detector) and internal calibration. Quantification is carried out via the isotope dilution method with individual response factors for each compound.
4.2	**Conclusion**	The validation results confirm that the method is suitable for the determination of Me-O-DCPP in water samples.
4.2.1	Reliability	2
4.2.2	Deficiencies	No

Section A4.2
Annex Point IIA, IV.4.2

Analytical Methods for Detection and Identification of Me-O-DCPP (metabolite of a.s.) in Water

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	*September 2013*
Materials and methods	Section 3.6:only the detection limit for the extract solution is given. Since there no sample amounts corresponding to the given extract volumes are given it is not possible to calculate detection limit as ng per ml sample
Conclusion	*Agree with applicants version with the amendments given above*
Reliability	*2*
Acceptability	*Acceptable*
Remarks	-

Section A4.3 **Analytical Methods for Detection and Identification**

BPD Annex Point IIIA, IV.1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN/ON FOOD OR FEEDSTUFFS

Official use only

1 REFERENCE

1.1	**Reference**	[redacted], 2008, Analysis of the DCPP in fatty food stimulant-sunflower oil. [redacted] KBB-544 (unpublished), Date: 2008-03-13
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No, no guideline available
2.2	**GLP**	No
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Preliminary treatment**	
3.1.1	Enrichment	No enrichment or clean-up is necessary. The sample solution should be vigorously shaken and cooled down to room temperature. The sample is then diluted 2 times in n-hexane. After homogenisation the mixture is analysed according to the indicated conditions.
3.1.2	Cleanup	
3.2	**Detection**	
3.2.1	Separation method	Chromatographic conditions: Column: YMC ProC18, 5 µm; 250 x 2.1 mm Oven temp.: 40 °C Mobile phase: A: AcetonitrileWater 70/30 B: THF Gradient elution Flow: 0.4 mL/min Injection: 10 µL
3.2.2	Detector	UV detector (wavelength: 210 nm)
3.2.3	Standard(s)	External standard DCPP (purity: > 99%).
3.2.4	Interfering substance(s)	Substances of specimen matrix may interfere.
3.3	**Linearity**	
3.3.1	Calibration range	10 concentrations in the range of 20 µg/L to 1000 µg/L were analysed.
3.3.2	Number of measurements	Each concentration was measured once.

Section A4.3 **Analytical Methods for Detection and Identification**
BPD Annex Point IIIA, IV.1 ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN/ON FOOD OR FEEDSTUFFS

3.3.3	Linearity	Correlation coefficient: 0.9999
3.4	**Specificity: interfering substances**	No interferences were observed.
3.5	**Recovery rates at different levels**	Samples of sunflower oil were fortified with 50 µg/L, 100 µg/L and 150 µg/L of active substance. At each fortification level five samples from five different weights were prepared. The recoveries found for samples fortified with 50 µg/L ranged from 87.2% to 92.5% and recoveries found for samples fortified with 100 µg/L ranged from 93.9% to 97.6%. The recoveries found for samples fortified with 150 µg/L were between 95.8% and 96.2%. The mean recoveries were 89.6% for the 50 µg/L fortification level, 94.8% for the 100 µg/L fortification level and 96.0% for the 150 µg/L fortification level.
3.5.1	Relative standard deviation	The relative standard deviations were 2.2% for the 50 µg/L fortification level, 1.7% for the 100 µg/L fortification level and 2.2% for the 150 µg/L fortification level.
3.6	**Limit of determination**	20 µg/L
3.7	**Precision**	
3.7.1	Repeatability	Please refer to the results of the recovery experiments summarised above (point 3.5).
3.7.2	Independent laboratory validation	No independent laboratory validation is available.

4 APPLICANT'S SUMMARY AND CONCLUSION

4.1	**Materials and methods**	Residues of the active substance in sunflower oil samples are analysed by means of liquid chromatography using UV detection at 210 nm after dilution in n-hexane. Quantification is done using external standard calibration.
4.2	**Conclusion**	The analytical method was sufficiently validated with respect to specificity, linearity, precision and accuracy. The method is therefore suitable for the determination of DCPP residues in sunflower oil in the range of 20 µg/L to 2 mg/L.
4.2.1	Reliability	Reliability indicator: 2
4.2.2	Deficiencies	No

Section A4.3	**Analytical Methods for Detection and Identification**
BPD Annex Point IIIA, IV.1	ANALYTICAL METHOD FOR THE DETERMINATION OF ACTIVE SUBSTANCE RESIDUES IN/ON FOOD OR FEEDSTUFFS

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	April 2009
Materials and methods	Acceptable
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	-

Section A5 Effectiveness against target organisms and intended uses

Subsection (Annex Point)			Official use only
5.1	**Function (IIA5.1)**	Bactericide	
5.2	**Organism(s) to be controlled and products, organisms or objects to be protected (IIA5.2)**	--	
5.2.1	**Organism(s) to be controlled (IIA5.2)**	DCPP is active against major germ types (e.g. Gram positive and Gram negative bacteria). Disinfectant formulations containing DCPP have a broad efficacy against potentially harmful germs of practical relevance (bacteria), e.g. *Staphylococcus aureus, Staphylococcus epidermidis, and Escherichia coli.* DCPP has the capability to reduce the number of viable bacterial cells of relevant organisms. The MIC values obtained for different bacteria species confirmed the broad antimicrobial efficacy of the active ingredient DCPP. For details see attached **Summary Table A5.1** and Efficacy part of Doc. III-B of BPD dossier.	
5.2.2	**Products, organisms or objects to be protected (IIA5.2)**	Several biocidal applications are intended for DCPP: - Disinfectant: biocidal product types 1, 2, 4 - Preservative: biocidal product type 9	x
		As a disinfectant, DCPP is applied for human hygiene purposes (product type (PT) 1), to keep surfaces free of bacteria in private and public health areas (PT 2) and as dish-washing agent in the frame of PT 4.	x
		Organisms to be protected: Human in private areas and public health areas (PT 1 and PT 2). Objects to be protected: Private areas and public health areas (PT 2), food and feed areas (PT 4).	
5.3	**Effects on target organisms, and likely concentration at which the active substance will be used (IIA5.3)**		

Section A5 Effectiveness against target organisms and intended uses

5.3.1	**Effects on target organisms (IIA5.3)**	DCPP has the capability to reduce the number of viable bacterial cells of relevant organisms. The MIC values obtained for different bacteria species confirmed the broad antimicrobial efficacy of the active ingredient DCPP, see results in **attached table A5.1.**	
		In study summary for **section A5_3_1** (Effects on target organisms) the efficacy of different doses of DCPP contained in a disinfectant product can be observed.	
		Furthermore, tests were conducted with several disinfectant products intended for post-contamination treatment of hands (PT 1), or cleaning of surfaces (PT 2), or to reduce the proliferation of germs in objects in contact with food or feed (PT 4). The products tested contained different proportions of DCPP as active substance.	X
		In these tests, DCPP was tested towards bacteria to determine the percentage reduction of surviving colonies on agar plates in contact with the active substance or the corresponding formulation.	
		For more detailed information on the products confer the separated study summaries for **section 5.10 of Document III-B of dossier** (Effects of formulated product on target organisms).	
5.3.2	**Likely concentrations at which the A.S. will be used (IIA5.3)**		
	PT1	Human hygiene biocidal products: up to 0.2% DCPP	
	PT2	Private area and public health disinfectant: up to 0.2% DCPP	X
	PT4	Food and feed area disinfectants: up to 0.2% DCPP (dish washing agent is diluted further in water to a treatment solution containing 0.004% DCPP)	X
5.4	**Mode of action (including time delay) (IIA5.4)**	--	
5.4.1	**Mode of action**	Due to the high degree of similarity of DCPP with triclosan regarding the chemical structure, data on triclosan is given for the description of the mode of action of DCPP. It is expected that both substances will have comparable properties.	
		Triclosan has several mechanisms of action including membrane destabilization, inhibition of fatty acid synthesis, efflux mechanisms, and formation of biofilms. Based on a comprehensive literature review (Colipa, 2005) the available information can be summarised as follows:	
		The different mechanisms described below are dependent on organism type and level of triclosan, and at higher concentrations, it is likely that the non-specific mechanisms tend to prevail, resulting in lower risk of decreased susceptibility developing.	
		A. Membrane Destabilization	
		The perturbing effects of triclosan on membrane structures suggest that this molecule would alter membrane functions, affecting not only lipids, but also indirectly the proteins of the membrane, the functions	

Section A5 Effectiveness against target organisms and intended uses

of which are highly dependent on membrane structure (Lygre et al., 2003).

B. Inhibition of Fatty Acid Synthesis

Numerous studies have been conducted on the pathway of fatty acid synthesis resulting in a clearer understanding of this mechanism in the various bacteria. At sub-lethal concentrations triclosan has been shown to target ENR, the enoyl-acyl carrier protein (ACP) reductase (Fabl enzyme) or enzymes of similar function in both Gram negative and Gram positive bacteria.

The x-ray crystal structure of ENR from *Escherichia coli* has been determined (Roujeinikova et al., 1999; Stewart et al., 1999). Triclosan sits in the binding pocket of the enzyme and forms hydrogen bonds with the 2-hydroxyl group of the nicotinamide. The 2-hydroxyl group is crucial for antibacterial activity.

The enoyl-acyl carrier protein reductases have been described from *Bacillus subtilis* (Schujman et al., 2001), mycobacteria (Kremer et al., 2003; Rawat et al., 2003), and *Staphylococcus aureus* (Fan et al., 2002). In the study on *S. aureus*, it was concluded that overexpression of the FabI enzyme did not appear to be advantageous, since it appeared in only one of the 31 clinical strains tested. Presence of these reductases in various bacteria indicates that they may well be susceptible to triclosan.

C. Efflux Mechanisms

The efflux of potentially harmful molecules is a common defense mechanism in bacteria. The mechanism by which triclosan is effluxed from bacteria has been elucidated in *Pseudomonas aeruginosa* (Chuanchuen et al., 2002; Chuanchuen et al., 2003), E. coli (Levy, 2002), *Salmonella enteria* (Braoudaki and Hilton, 2005), and *Stenotrophomonas maltophilia* (Sanchez et al., 2005). The up-regulation of the efflux mechanism by sublethal levels of triclosan was observed in several investigations. (Levy, 2002; Sanchez et al., 2005, Chuanchuen et al., 2002).

D. Biofilms

Two studies using oral plaque biofilms have shown that, as expected, triclosan was less effective against organisms in biofilms when compared to the same organisms in planktonic suspensions (Fine et al., 2001; McBain et al., 2003a). The mean MIC of triclosan for the biofilm strains did not change significantly after five (5) days of treatment (McBain et al., 2003a).

5.4.2 Time delay

Not relevant, since the biocidal effect of the active substance starts immediately after application.

5.5 Field of use envisaged (IIA5.5)

MG01: Disinfectants, general biocidal products

PT 1: Human hygiene biocidal products

PT 2: Private area and public health area disinfectants

PT 4: Food and feed area disinfectants

MG02: Preservatives

MG03: Pest control

Section A5 Effectiveness against target organisms and intended uses

	MG04: Other biocidal products Further specification		
5.6	**User (IIA5.6)**		
	Industrial	Not relevant	
	i) Open system	-	
	ii) Closed system		
	Professional	Product is intended to be used professionals (PT 1, 2, and 4): For details see Documents II-B and II-C of BPD dossier.	
	i) Open system	All disinfectants are used in open systems.	
	ii) Closed system		
	General public	Formulations according to PT 1, 2 and 4 are intended to be used also by general public. For details see Documents II-B and II-C of BPD dossier.	
5.7	**Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA5.7)**		
5.7.1	**Development of resistance**	Development of resistance due to the biocidal use of DCPP is regarded as not relevant. Three comprehensive reviews are available for Triclosan, i.e. a substance with high degree of similarity to DCPP: 1) The text of the **European Commission (2002)** presents the opinion of the Scientific Steering Committee (SSC) as requested by the European Commission on the possible impact of the use of DCPP on the development of antimicrobial resistance. The conclusions of the report were as follows: At high (biocidal) concentrations, DCPP is very effective and unlikely to produce a major problem of anti-microbial resistance. However, at sub-biocidal and bacteriostatic, concentrations, DCPP is capable of penetrating bacteria and initiating changes related to important mechanisms of antimicrobial resistance including possibly transferable mechanisms of resistance, though the scientific evidence for transferability has been disputed. Sound scientific laboratory evidence exists for the development of DCPP related mechanisms for antimicrobial resistance, but the evidence as to whether these mechanisms are shared by other antimicrobial agents or whether they are transferable to micro-organisms other than those used in the laboratory is limited and contradictory. No evidence of such resistance has been seen so far in clinical isolates, and there is no epidemiological evidence to suggest a problem in clinical practice. There are, however, very few targeted studies of resistance to DCPP in relevant clinical or wider environments. Although, the stability and persistence of DCPP resistance has not	x

Section A5 Effectiveness against target organisms and intended uses

been widely studied, the limited information available points to it being stable over a three to ten year period.

Thus, there is no convincing evidence that DCPP poses a risk to humans or to the environment by inducing or transmitting antibacterial resistance under current conditions of use.

Due to the high degree of similarity of DCPP with Triclosan regarding the chemical structure, it is expected that both substances will have similar properties. Additional data on Triclosan is given in the following for the description of resistance of DCPP:

2) In the **Colipa (2005)** literature review the available literature is summarised as follows:

Considering that Triclosan has a history of use of over thirty (30) years, it is highly likely that if bacteria were going to develop Triclosan resistance, such bacteria would already be evident in the environments where Triclosan-containing products have been repeatedly used. Surveillance studies searching for Triclosan-resistant bacteria in the Triclosan factory, in clinical settings, in the home, on the skin or in the oral cavity of users of Triclosan have not found any such organisms. While the surveillance studies can be criticized for being limited in scope, it is affirming that in all of the environmental surveys published to date, no evidence of increasing resistance to Triclosan has been shown. While these findings do not preclude the possibility that Triclosan resistance can develop outside the laboratory, they indicate that such a development does not commonly or readily occur. They also indicate that intrinsically resistant species do not out-compete susceptible strains in biocide-treated environments.

Laboratory studies have shown some cross-resistance to antibiotics and other biocides in some laboratory-derived mutants of *E. coli*, pseudomonads, and staphylococci. However, there is growing evidence that the generation of such mutants is strain specific and that these mutations do not appear to occur in all species. In fact there have been a number of reported failures by researchers trying to develop Triclosan-resistant strains (McBain et al., 2004). Additionally, such mutations are frequently lost when Triclosan is removed from the media, suggesting that there is no competitive advantage to the organism to conserve that mutation.

3) In the text by **Pickardt (2006)** two surveys on efficacy of Triclosan are cited that led to the following conclusions:

Environmental surveys have not demonstrated an association between Triclosan usage and antibiotic resistance (Cole et al. 2003, Aiello et al. 2005). Both were randomized studies, comparing households of users and non-users of antibacterial products (Cole et al./n = 60, Aiello et al./n = 224). The surveys were relatively small, i.e. their significance should not be overstated. Interestingly, both groups draw different conclusions. Cole et al. comment, that their results “refute widely published, yet unsupported, hypotheses that use of antibacterial products facilitates the development of antibiotic resistance in bacteria from the home environment”, and agrees with a number of other experts (see e.g. Gilbert & McBain 2004, Percival et al. 2005) in that the risks associated with the overuse of biocides have been overstated.

Section A5 Effectiveness against target organisms and intended uses

		In contrast, Aiello et al. state: “Although we did not observe a significant impact on antimicrobial drug resistance during the 1-year period, a longer duration and more extensive use of triclosan might provide a suitable environment for emergence of antimicrobial drug–resistant species in the community setting; further surveillance ... in the community is needed”.
5.7.2	**Management strategies**	Not relevant since development of resistance is not expected
5.8	**Likely tonnage to be placed on the market per year (IIA5.8)**	This information is confidential and therefore provided separately in the confidential part of the dossier.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date July 2010 to May 2011

Materials and methods Acceptable with the amendments below

Results and discussion During the evaluation phase of this document the CA had a thorough discussion with the applicant. The elements of the discussion, sorted in chronological order, are given below. The arguments raised are considered to be useful for future product authorizations.

Section 5.2.2: PT9 is not relevant for this CAR

Sections 5.2.2 and 5.3.1

Comments CA (July 2010)

> *As a disinfectant, DCPP is applied for human hygiene purposes (product type (PT) 1), to keep surfaces free of bacteria in private and public health areas (PT 2) and as dish-washing agent in the frame of PT 4.*
>
> Please notice, that “to keep surfaces free of bacteria” means, that a surface contains an antimicrobial active substance!
>
> Please clarify if the DCPP (PT2) is used as a surface disinfectant or a surface cleaner or as an antimicrobial component of a treated material.

BASF response (Oct. 2010)

> DCPP is used as a PT2 biocide in surface cleaners and disinfectants. But due to DCCP's properties, the surface is not just disinfected during application of the DCCP-containing product but the treated surface also provides a long-term antimicrobial activity which is shown by AATCC 100-1999 studies and diffusion test methods.

Section 5.3.2

Comments CA (July 2010)

> *PT2Private area and public health disinfectant: up to 0.2% DCPP*
>
> *PT4Food and feed area disinfectants: up to 0.2% DCPP (dish washing agent is diluted further in water to a treatment solution containing 0.004% DCPP)*
>
> This is not correct. According to the “General Information on the Active Substance” and the documents B5_PT2_DCPP or B5_PT4_DCPP respectively, the dilution factor is 1:50 or 1:500 corresponding to 0.004% (PT2) or 0.0004% (PT4) respectively.

BASF response (Oct. 2010)

> The concentrations used in the formulations tested for PT 2 were between 0.09 and 0.2% DCPP. Various dilutions of the DCPP-containing formulations have been tested (see A5_DCPP_BASF_Attach 1_rev 102010.xls) showing activity at use concentrations down to 0.00015% DCPP for PT2 uses and 0.00006% DCPP for PT4 uses.

	Section 5.7.2: As resistance and cross-resistance cannot be excluded a resistance management strategy is relevant. See also list of available literature on resistance given in Doc IIB 2.15. CA Summary (July 2010) For the inclusion of the active substance DCPP as PT1, PT2 or PT4, efficacy tests according to the European phase 2 / step 1 and phase 2/ step 2 Norms and/or the Standard methods of the German Society of Hygiene and Microbiology are necessary! The submitted test reports of Hoffstetter 2005 and 2007 are not adequate (see table A5-1 and references). BASF response to CA summary (Oct. 2010) BASF will submit EN1040 study (Phase 1 norm) to support activity of the active substance DCPP (Part A). BASF will also submit EN1276 studies (Phase 2/step 1 norm) to support activity of formulations containing DCPP in PT2 and PT4.
Conclusion	BASF has shown bactericidal activity for DCPP in phase 1 and phase 2/step 1 tests (see A5.3.1_DCPP_EN 1040_BASF_122010 and A5.3.1_DCPP_EN 1276_BASF_122010). Additionally, tests according to the mentioned norms have been performed with Propylene Glycol, which is the carrier in [redacted] (test product in Hoffestetter 2005), and it did not show any significant reduction of the test organisms within time span, therefore the bactericidal activity is due to DCPP. Please note that EN 1276 is the required test for evaluation the bactericidal activity of chemical disinfectants used in food, industrial, domestic and institutional areas. For products based on DCPP, phase 2/step 2 tests (according to national or international guidelines or norms) should be performed by the companies who will register each of their DCPP-containing formulations as **Biocidal Products** once DCPP is listed on Annex 1, as a biocide, to show their activities under practical conditions (i.e soiling conditions).
Reliability	2 (reliable with restrictions)
Acceptability	Acceptable with the amendments given above
Remarks	-

Table A5-1: Summary Table: Data available on the effectiveness of DCPP as disinfectant

Test substance	Test organism(s)	Test system / concentrations applied / exposure time	Test results: lowest non-preventive concentration	Reference
DCPP	*Staphylococcus aureus* ATCC 6538 *Staphylococcus epidermidis* ATCC 12228 *Enterococcus hirae* ATCC 10541 *Corynebacterium minutissimum* ATCC 23348 *Escherichia coli* ATCC 10536 *Pseudomonas aeruginosa* ATCC 15442 *Candida albicans* ATCC 10231 *Aspergillus niger* ATCC 6275	Determination of MIC values by the Agar incorporation method (CIBA internal method CG 128e). Concentrations in the agar plates were 100 µg/ml / 50 µg/ml / 10 µg/ml / 5 µg/ml / 1 µg/ml / 0.5 µg/ml / 0.1 µg/ml / 0.05 µg/ml / 0.01 µg/ml / 0.005 µg/ml. Incubation time: 24 to 48 hours	*Staphylococcus aureus* 0.05 ppm, *Staphylococcus epidermidis* 0.05 ppm, *Enterococcus hirae* 50 ppm, *Corynebacterium minutissimum* 10 ppm, *Escherichia coli* 0.5 ppm, *Pseudomonas aeruginosa* > 100 ppm, *Candida albicans* 10 ppm, *Aspergillus niger* 10 ppm	[redacted] 2007
Floor cleaner containing 0.09-0.18% DCPP	*Staphylococcus aureus* ATCC 6538 *Salmonella choleraesuis* ATCC 10708 The germs chosen for the test represent pathogenic Gram-positive and Gram-negative bacteria playing an important role in general hygiene.	**Test method:** Modified AATCC test method 100-1998 (Assessment of the antibacterial finishes on textile materials) **Dosage rate:** Four solutions tested: 0.3, 0.4, 0.5, 0.6% [redacted] i.e. 0.09, 0.12, 0.15, 0.118% DCPP. **Application procedure:** 8 flooring material discs (20 mm diameter) were given in 20 mL floor cleaner formulation (2% formulation solution) and treated for 2 minutes. **Test procedure:** Test and control (water only without [redacted] discs were inoculated with the test organisms. The number of bacteria present is determined and the percentage reduction by the treated specimen is calculated. Incubation: 24 hours at 37°C on test plates	A bactericidal activity of 1.3 – 2.8 $\log_{10}$ against the Gram-positive *Staphylococcus aureus* ATCC 6538 has been achieved 24 hours after treatment with the floor cleaner formulations containing 0.3% - 0.6% [redacted] at an in-use concentration of 2% in comparison to material treated with water only. Therefore, a clear dose-response relationship was observed. Against the Gram-negative *Salmonella choleraesuis* ATCC 10708 no satisfactorily activity could be shown (< 1log reduction after 24 hours contact time) at an in-use concentration of 2%. Conclusion: The active substance DCPP in T[redacted] provided substantial protection against *Staphylococcus aureus* but not against *Salmonella choleraesuis*.	[redacted] 2005

References

Colipa (2005): Submission by Colipa to the EU, September 2005. Literature review on bactericidal resistance and Triclosan between 2002 and 2005 (published).

European Commission (2002): Opinion on DCPP Resistance. Adopted by the Scientific Steering Committee at its meeting of 27-28 June 2002. European Commission, Health and consumer protection directorate-general. Directorate C – Scientific Opinions, C1 – Follow-up and dissemination of scientific opinions (published).

[redacted](2005): Determination of the bactericidal activity of 4 antibacterial floor cleaners containing Ciba®[redacted]on treated flooring material. Ciba Specialty Chemicals, Lab. Journal No. 120 Page 11, Technical Report No.[redacted] date: 2005-08-15 (unpublished).

[redacted](2007): Determination of the Minimal Inhibitory Concentration (MIC) of[redacted]® DCPP (5-chloro-2-(4-chlorphenoxy)phenol). Ciba Specialty Chemicals, Lab. Journal No. 120 Page 82, Technical Report No.[redacted] date: 2007-04-20 (unpublished).

[redacted](2006): Background paper "Considering the potential of resistance in the efficacy and risk evaluation of biocidal compounds" (based on the TMIII 05 discussion to OECD Thought Starter from October 2005). On behalf of the Federal Institute for Risk Assessment, Berlin, Germany (BfR), Document name: TMI06GEN-item14b-resistence-in-target-organinsms.doc, date: 2006-03-01 (published).

[redacted]

[redacted]

[redacted]

3.2 [redacted]

Product: Stock Solution Test concentration: 2000 ppm		
Contact time / Test strain	*S. aureus*	*P. aeruginosa*
5 min	> 5.36	> 5.33
Test concentration: 200 ppm		
5 min	> 5.36	> 5.33
Test concentration: 20 ppm		
5 min	< 1.29	1.81

[redacted]

Product: Propylene Glycol Stock Solution Test concentration: 13.13%		
Contact time / Test strain	*S. aureus*	*P. aeruginosa*
5 min	< 1.29	< 1.26

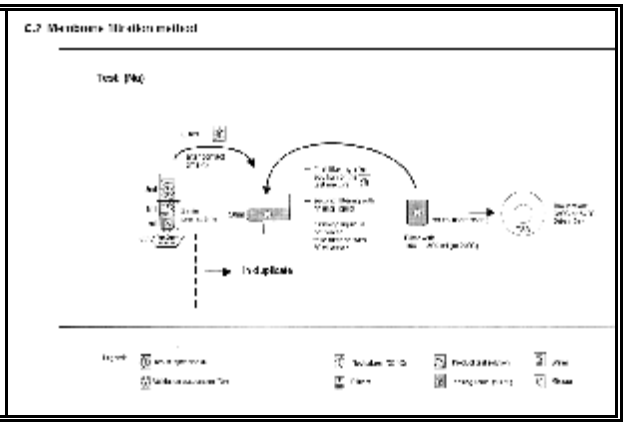
[redacted]

[redacted]

[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

1.2

	pots

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]

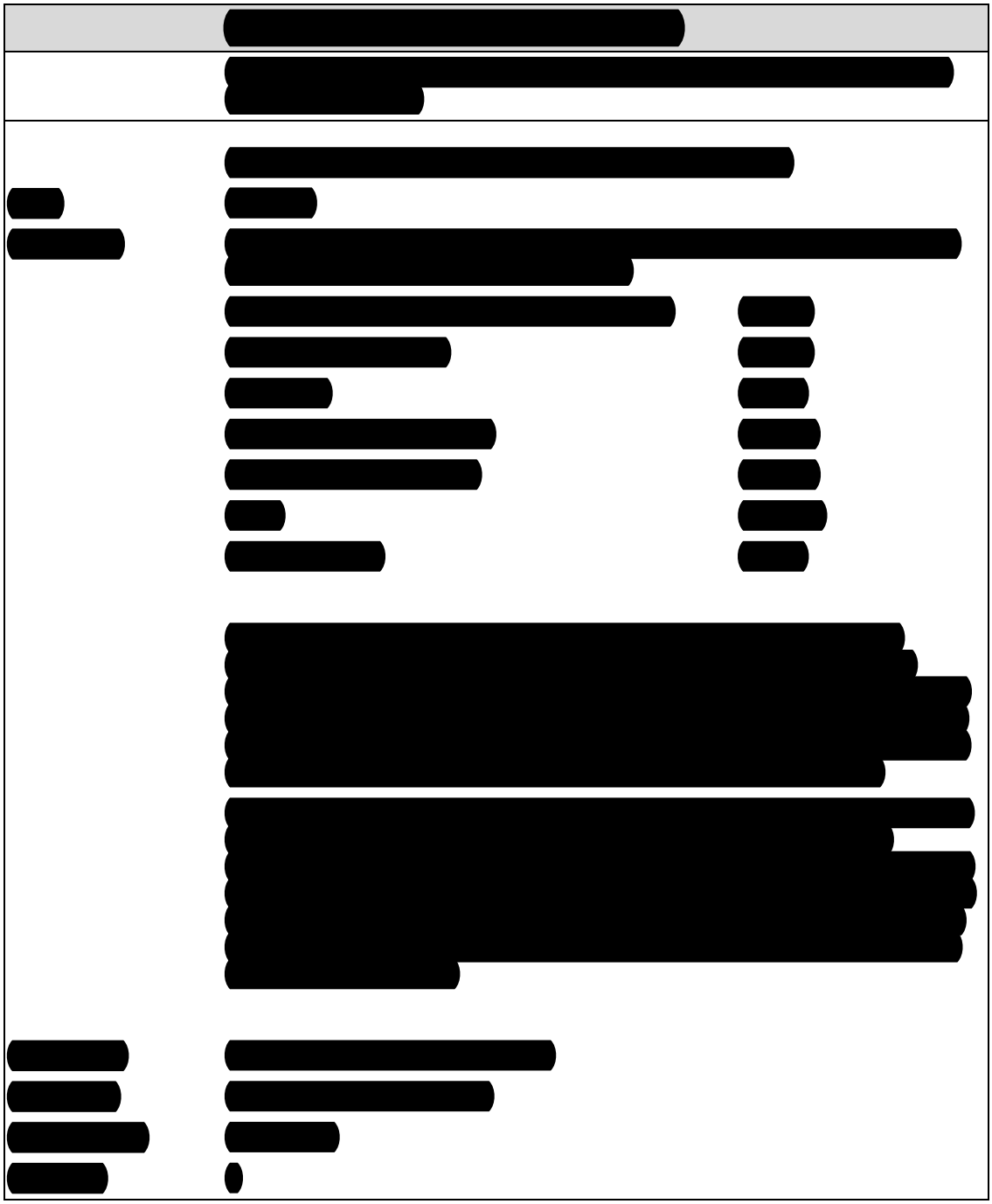
2.3.1 [redacted]

[redacted]

Product: AM10/032/04 Test concentration: 80% (= undiluted) Loading: 0.03% albumin				
Contact time / Test strain	*S. aureus*	*E. coli*	*Ec. hirae*	*P. aeruginosa*
5 min	> 5.36	> 5.35	> 5.42	> 5.33

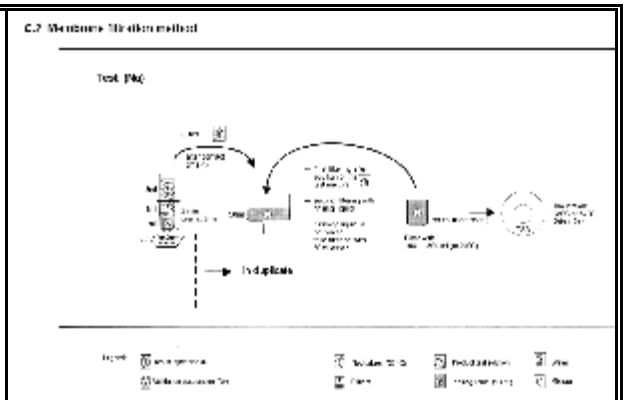
[redacted]

4.3.3



[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

1.2 [redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]	[redacted]

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (ATC test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1999a), [redacted] Acute Oral Toxicity Study in Rats. [redacted] Project No. 712080, date: 1999-01-08 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline 423 (1996) = EC Method B.1 tris (1996)
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Solid powder
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in vehicle: 10 days (1% in polyethylene glycol)
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	HanIbm: WIST (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	♂: 8 weeks, 201-211 g ♀: 10 weeks, 179-189 g
3.2.6	Number of animals per group	3 per sex
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Post-exposure period	14 days
3.3.2	Type	Gavage

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (ATC test)

3.3.3	Dose	2000 mg/kg bw
3.3.4	Vehicle	Polyethylene glycol
3.3.5	Concentration in vehicle	0.2 g/mL
3.3.6	Administered volume	10 mL/kg bw
3.3.7	Control	–
3.4	**Examinations**	Mortality / Viability: four times during test day 1, once daily during days 2-15 Clinical observations: four times during test day 1, once daily during days 2-15 Body weights: pre-test and on Days 8 and 15 Necropsy: on day 15
3.5	**Method of determination of LD_{50}**	ATC method flow chart
3.6	**Further remarks**	None

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	No death occurred during the study. Only one male showed diarrhoea five hours after treatment. All other animals were free of clinical signs.
4.2	**Pathology**	No macroscopic findings were observed at necropsy.
4.3	**Other**	-
4.4	**LD_{50}**	LD_{50} > 2000 mg/kg bw

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Two groups, each using three female or three male Hanlbm: WIST (SPF) rats, were treated with [redacted] 2000 mg/kg by oral gavage. The test article was suspended in vehicle (polyethylene glycol) at a concentration of 0.2 g/mL and administered at a volume of 10 mL/kg bw. The animals were examined for clinical signs four times during test day 1 and once daily during test days 2-15. Mortality/viability was recorded together with clinical signs at the same time intervals. Body weights were recorded on day 1 prior to administration and on days 8 and 15. All animals were necropsied and examined macroscopically.
5.2	**Results and discussion**	No death occurred during the study. One male showed diarrhoea five hours after treatment only. No clinical signs were observed in all females and in the two remaining males during the observation period. The body weight of the animals was within the range commonly recorded for animals of this strain and age. No macroscopic findings were observed at necropsy.

Section A6.1.1 Acute Toxicity

Annex Point IIA VI.6.1.1 6.1.1 Acute oral toxicity in rats (ATC test)

5.3	**Conclusion**	The acute oral LD_{50} of DCPP in the rat is greater than 2000 mg/kg bw. No classification for acute oral toxicity is warranted.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2011
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit test)

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1999b), [redacted] Acute Dermal Toxicity Study in Rats. [redacted] Project No. 712091, date: 1999-01-12 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline 402 (1987) = EC Method B.3 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted] DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Solid powder
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in vehicle: 10 days at RT (1% in polyethylene glycol)
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	HanIbm: WIST (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	♂: 9 weeks, 234-259 g ♀: 11 weeks, 208-216 g
3.2.6	Number of animals per group	5 per sex
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Dermal
3.3.1	Post-exposure period	14 days
3.3.2	Area covered	10% of body surface
3.3.3	Occlusion	Semi-occlusive

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit test)

3.3.4	Vehicle	Polyethylene glycol
3.3.5	Concentration in vehicle	0.5 g/mL
3.3.6	Total volume applied	4 mL/kg bw
3.3.7	Applied doses	2000 mg/kg bw
3.3.8	Duration of exposure	24 hours
3.3.9	Removal of test substance	With tepid water.
3.3.10	Control	None
3.4	**Examinations**	Mortality / Viability: four times during test day 1, once daily during days 2-15 Clinical observations: four times during test day 1, once daily during days 2-15 Body weights: pre-test and on Days 8 and 15 Necropsy: on day 15
3.5	**Method of determination of LD_{50}**	not applicable
3.6	**Further remarks**	-

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	No deaths occurred during the study. Slight scaling of the treated skin was observed in all animals. All males and four females showed focal erythema. In three males and in two females, serous rhinorrhoea was noted. The treated skin was crusted in one animal only. All clinical and local signs were reversible after 15 days.
4.2	**Pathology**	No macroscopic findings were observed at necropsy.
4.3	**Other**	The body weight of the animals was within the range commonly recorded for animals of this strain and age.
4.4	**LD_{50}**	LD_{50} > 2000 mg/kg bw

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A group of five male and five female Hanlbm: WIST (SPF) rats was treated with [redacted] at 2000 mg/kg by dermal application. The test article was suspended in vehicle (polyethylene glycol) at a concentration of 0.5 g/mL and administered at a volume of 4 mL/kg. The animals were examined for clinical signs four times during day 1 and once daily during days 2-15. Mortality/viability were recorded together with clinical signs at the same time intervals. Body weights were recorded on day 1 prior to administration and on days 8 and 15. All animals were necropsied and examined macroscopically.

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit test)

5.2	**Results and discussion**	No deaths occurred during the study. Slight scaling of the treated skin was observed in all animals. All males and four females showed focal erythema In three males and in two females, serous rhinorrhoea was noted. The treated skin was crusted in one animal only. All clinical and local signs were reversible after 15 days. The body weight of the animals was within the range commonly recorded for animals of this strain and age. No macroscopic findings were observed at necropsy.
5.3	**Conclusion**	The acute percutaneous LD_{50} of DCPP in the rat is greater than 2000 mg/kg bw. No classification for this endpoint is warranted.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A6.1.2 Acute Toxicity

Annex Point IIA VI.6.1.2 6.1.2 Acute dermal toxicity in rats (Limit test)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2011
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section 6.1.3 **Annex Point IIA VI.6.1.3**	**Acute toxicity** **Acute inhalation toxicity study in rats**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	An acute inhalation study with DCPP has not been conducted. Exposure towards DCPP by inhalation is negligible compared to the main route of exposure which is direct dermal contact. The vapour pressure of DCPP at 25°C is only 1.2×10^{-06} Pa, thus exposure to DCPP vapours is considered negligible. Furthermore, the intended use of DCPP does not involve application by spraying or any other technique that might generate respirable aerosols. As a conclusion, inhalation is not a relevant route of exposure to DCPP and animal studies addressing inhalation toxicity of DCPP are not necessary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4(1) Acute dermal irritation

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]A: Primary Skin Irritation Study in Rabbits (4-Hour Semi-Occlusive Application). [redacted], Project No. 712102, date: 1998-12-22 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 404 (1992) = EC Method B.4 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted]= DCPP	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	Solid powder	
3.1.2.2	Purity	> 99%	
3.1.2.3	Stability	Expiration date: October 31, 2008 Stable at storage conditions	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	Age: 15 weeks Weight: 2806-2987 g	
3.2.6	Number of animals per group	1 male, 2 females	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Application		
3.3.1.1	Preparation of test substance	0.5 g of the test substance was moistened with bidistilled water.	
3.3.1.2	Test site and preparation of test site	Dorsal fur (10 × 10 cm²) was clipped with an electric clipper three days before treatment..	

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4(1) Acute dermal irritation

3.3.2	Occlusion	Semi-occlusive
3.3.3	Vehicle	Moistened with water
3.3.4	Removal of test substance	With tepid water
3.3.5	Duration of exposure	4 h
3.3.6	Post-exposure period	72 h
3.3.7	Controls	none
3.4	**Examinations**	
3.4.1	Clinical signs	Yes, daily.
3.4.2	Dermal examination	
3.4.2.1	Scoring system	According to Draize scoring system. Erythema 0-4: 0: No erythema, 1: very slight erythema (barely perceptible), 2: well-defined erythema, 3 moderate to severe erythema, 4: severe erythema (beet redness) to slight eschar formation (injuries in depth) Oedema 0-4: 0: No oedema, 1: very slight oedema (barely perceptible), 2: well-defined oedema (edges of area well-defined by definite raising), 3: moderate to severe oedema (raised approximately 1mm), 4: severe oedema (raised more than 1 mm extending beyond the area of exposure)
3.4.2.2	Examination time points	At 1, 24, 48, 72 h after removal of the test patch.
3.4.3	Other examinations	Body weights were determined at start of acclimatization, on the day of application and at termination.
3.5	**Further remarks**	None
		4 RESULTS AND DISCUSSION
4.1	**Average score**	There were no signs of irritation on any rabbit at any of the examination time points.
4.1.1	Erythema	0.0
4.1.2	Oedema	0.0
4.2	**Reversibility**	–
4.3	**Other examinations**	–
4.4	**Overall result**	Not irritating.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The primary skin irritation potential of the test article was investigated by topical application of 0.5 g DCPP to 6 cm² intact dorsal skin of each of three young adult New Zealand White rabbits. The duration of treatment was four hours. The scoring of skin reactions was performed 1, 24, 48 and 72 hours after removal of the dressing. The scores of each animal at the following reading times (24, 48, 72 hours) were used in calculating the respective mean values for each type of lesion.

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4(1) Acute dermal irritation

5.2	**Results and discussion**	Local signs (mean values from 24 to 72 hours) consisted of grade 0.00 erythema and grade 0.00 oedema. No signs of irritation were observed. The test article did not cause staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any measuring interval.
5.3	**Conclusion**	Based upon the criteria of Directive 2001/59/EC, the test article is considered to be “not irritating” to rabbit skin.
5.3.1	Reliability	1
5.3.2	Deficiencies	None.

Section A6.1.4 Acute Dermal Irritation

Annex Point IIA VI.6.1.4 6.1.4(1) Acute dermal irritation

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2011
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4(2) Acute eye irritation

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](1999),[redacted] Primary Eye Irritation Study in Rabbits. [redacted] Project No. 712113, date: 1999-01-29 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 405 (1987) = EC Method B.5 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Solid powder
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	New Zealand White
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	Age: 15 weeks Weight: 2890-3200 g
3.2.6	Number of animals per group	1 male, 2 females
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Ocular instillation
3.3.1	Preparation of test substance	Test substance was used as delivered.
3.3.2	Amount of active substance instilled	0.1 g

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4(2) Acute eye irritation

3.3.3	Exposure period	Eyes were not rinsed			
3.3.4	Post-exposure period	21 days			
3.4	**Examinations**				
3.4.1	Ophthalmoscopic examination	Yes			
3.4.1.1	Scoring system	Grades of ocular lesions: Cornea 0 – 4 (0 = no finding, 1 = slight, disperse, diffuse opacity, 2 = extensive, diffuse opacity, iris blurred, 3 = mother-of-pearl-like opacity, iris and pupil hardly recognisable, 4 = complete opacity, ulceration) Iris 0 – 2 (0 = no finding, 1 = swelling, reddening, positive light reaction, 2 = severe reddening and swelling, no light reaction) Conjunctivae Redness 0 – 3 (0 = blood vessels normal, 1 = vessels abnormally filled, 2 = diffuse reddening, 3 = diffuse deep reddening) Swelling 0 – 4 (0 = no swelling, 1 = slight swelling, 2 =severe swelling, lids everted, 3 = lids cover one half of eye, 4 = lids cover more than half eye, necroses and ulcers on the conjunctivas)			
3.4.1.2	Examination time points	1 h, 24 h, 48 h, 72 h, 7 d, 14 d, 21 d			
3.4.2	Other examinations	None			
3.5	**Further remarks**	–			
		4 RESULTS AND DISCUSSION			X
4.1	**Average score**	**Rabbit #1 (♂)**	**Rabbit #2 (♀)**	**Rabbit #3 (♀)**	
4.1.1	Cornea	0.00	0.67	0.67	
4.1.2	Iris	0.00	0.00	0.00	
4.1.3	Conjunctiva				
4.1.3.1	Redness	2.00	1.00	2.00	
4.1.3.2	Chemosis	2.00	1.00	2.00	
4.2	**Reversibility**	Grade 1 corneal opacity was still persistent in Rabbit #2 after 21 days. Grade 1 conjunctival redness was still persistent in Rabbits #2 and #3 after 21 days.			
4.3	**Other**	–			
4.4	**Overall result**	The test substance is strongly irritating (R41) to the eyes of rabbits.			

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4(2) Acute eye irritation

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The primary irritation potential of the test article was investigated by instillation of 0.1 g into one eye of each of three young adult New Zealand White rabbits.

The treated eyes were not rinsed after application.

Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7, 14 and 21 days after test article application. The scores of each animal at the following reading times (24, 48 and 72 hours) were used in calculating the respective mean values for each type of lesion.

5.2 Results and discussion

The primary irritation score was calculated by totalling the individual cumulative scores at 24, 48 and 72 hours and then dividing the resulting total by the number of data points. The primary irritation score was 3.78 (max. 13).

In all animals, reddening (including hyperaemia of the scleral blood vessels) and swelling of the conjunctivae, swelling of the nictitating membrane, as well as watery discharge was observed.

In two animals, slight opacity arid mucous discharge was evident. Hyperaemia of the scleral blood vessels persisted in all animals until the end of the observation period. In two animals, conjunctival reddening and in one animal, opacity was noted until 21 days after treatment.

No staining of the treated eyes by the test article was observed.

No corrosion was observed at any of the measuring intervals.

5.3 Conclusion

DCPP is strongly irritating to eyes. The criteria for classification with **Xi, R41 - Risk of serious damage to eyes** are met**.**

5.3.1 Reliability 1

5.3.2 Deficiencies None

Section A6.1.4 Acute Eye Irritation

Annex Point IIA VI.6.1.4 6.1.4(2) Acute eye irritation

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2011
Materials and Methods	Agree with applicant's version
Results and discussion	Addition 4.1: Average Score – mean value of 3 time points (24h, 48h, 72h)
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	-

Table A6_1_4E-1. Results of eye irritation study

Animal Number	Sex	Evaluation Interval	Corneal Opacity	Iris	Conjunctivae		Cumulative	
					Redness	Chemosis	Score	Mean
1	m	1 hour	0	0	1	0	1.00	2.00
2	f		0	0	1	1	2.00	
3	f		0	0	1	2	3.00	
1	m	24 hours	0	0	2	3	5.00	4.00
2	f		0	0	1	1	2.00	
3	f		0	0	2	3	5.00	
1	m	48 hours	0	0	2	2	4.00	4.00
2	f		1	0	1	1	3.00	
3	f		1	0	2	2	5.00	
1	m	72 hours	0	0	2	1	3.00	3.33
2	f		1	0	1	1	3.00	
3	f		1	0	2	1	4.00	
1	m	7 days	0	0	1	0	1.00	2.33
2	f		1	0	1	1	3.00	
3	f		1	0	1	1	3.00	
1	m	14 days	0	0	0	0	0.00	1.00
2	f		1	0	1	0	2.00	
3	f		0	0	1	0	1.00	
1	m	21 days	0	0	0	0	0.00	1.00
2	f		1	0	1	0	2.00	
3	f		0	0	1	0	1.00	

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Guinea Pig Maximisation Test

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](1999), [redacted]A: Contact Hypersensitivity in Albino Guinea Pigs – Maximisation Test. [redacted] Project No. 712124, date: 1999-02-22 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD 406 (1992) = EC Method B.6 (1996)
2.2	**GLP**	Yes
2.3	**Deviations**	OECD 406 recommends extending the number of test animals to 20 treated/10 controls in case of a negative test. This was not done.
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Pale beige powder
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stable in PEG 1% for at least 10 days at RT.
3.1.3	Preparation of test substance for application	Test item was formulated in PEG 400
3.1.4	Pre-test performed on irritant effects	Yes
3.2	**Test Animals**	
3.2.1	Species	Guinea pig
3.2.2	Strain	Ibm: GOHI (SPF) ≅ Himalayan spotted
3.2.3	Source	[redacted]
3.2.4	Sex	♀
3.2.5	Age/weight at study initiation	4-6 weeks, 306-396 g
3.2.6	Number of animals per group	Test group: 10 Neg. control group: 5
3.2.7	Control animals	Yes, negative + positive

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Guinea Pig Maximisation Test

3.3	**Administration/ Exposure**	Guinea pig maximisation test
3.3.1	Induction schedule	Day 1: Intradermal induction Day 8: Topical induction
3.3.2	Duration of topical induction exposure	48 h
3.3.3	Way of induction	Day 7: pre-treatment with 10% SLS Day 8: epidermal application to the scapular area
3.3.4	Vehicle	PEG 400
3.3.5	Application volumes	Intradermal: 0.1 mL/injection site Topical: 0.3 mL
3.3.6	Concentrations used for induction	Intradermal: 5% Topical: 50%
3.3.7	Challenge schedule	Day 22
3.3.8	Duration of challenge exposure	24 h
3.3.9	Concentrations used for challenge	50%
3.3.10	Rechallenge	No
3.3.11	Scoring schedule	24 and 48 hours after the challenge application
3.3.12	Scoring system	0 - no reaction 0.5 - very faint erythema, usually non-confluent 1 - faint erythema usually confluent 2 - moderate erythema 3 - severe erythema with or without oedema
3.3.13	Removal of the test substance	Yes
3.3.14	Positive control substance	2-Mercaptobenzothiazole was tested from 1998-12-01 through 1999-01-08. The test on DCPP was performed from 1998-12-14 through 1999-01-08.
3.4	**Examinations**	Viability/mortality: once daily Clinical signs (local/systemic): once daily Skin reactions (erythema) during pre-test, induction and challenge Pre- and post-test body weights
3.4.1	Pilot study	Pre-test on intradermal application on one guinea pig with 1%, 3% and 5% DCPP in PEG 400. Pre-test on topical application on two guinea pigs with 10%, 15%, 25% and 50% DCPP in PEG 400.
3.5	**Further remarks**	–
		4 RESULTS AND DISCUSSION
4.1	**Results of pilot studies**	Intradermal injection of 5% DCPP caused grade 1 oedema/erythema, whereas topical application of 50% DCPP was not irritating.

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Guinea Pig Maximisation Test

4.2	**Results of test**	see Table A6_1_5-1
4.2.1	24 h after challenge	Positive reactions: Test group: 1/10 Naïve control group: 0/5
4.2.2	48 h after challenge	Positive reactions: Test group: 1/10 Naïve control group: 0/5
4.2.3	Other findings	–
4.3	**Overall result**	The test substance is not sensitizing according to the criteria for an adjuvant test (≥ 30% sensitisation).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	In order to assess the cutaneous allergenic potential of [redacted] the Maximization-Test was performed in 15 (10 test and 5 control) female albino guinea pigs, in accordance with OECD Guideline No. 406. The intradermal induction of sensitization was performed with a 5% dilution of the test article in PEG 400 and in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitization was conducted under occlusion with the test article at 50% in PEG 400. Two weeks after the epidermal induction application the challenge was completed by epidermal application of the test article at 50% in PEG 400 under occlusive dressing. The animals of the control group were induced with PEG 400 and FCA/physiological saline and challenged similarly to those of the test group. Cutaneous reactions, i.e. erythema and eschar, as well as oedema formation were evaluated at 24 and 48 hours after removal of the dressing. 2-Mercaptobenzothiazole (10% in mineral oil) was used as a positive control substance in a reliability test
5.2	**Results and discussion**	One DCPP-induced animal displayed erythema at the site of DCPP challenge at the 24- and 48-h scoring time. All other animals of the test substance group were free of skin reactions towards DCPP challenge. The positive control, 2-mercaptobenzothiazole, elicited a positive response in 10/10 test animals.
5.3	**Conclusion**	DCPP does not fulfil the criteria of Directive 2001/59/EC for classification as a skin sensitizer (≥ 30% sensitized animals in an adjuvant test).
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Guinea Pig Maximisation Test

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2011
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable for risk assessment Although the study has a major deviation from the OECD protocol (although the result was negative, the number of tested animals was not extended to 20 treated/10 controls) the study is well conducted and allows the evaluation of the sensitising potential of DCPP. The tested concentration was relatively high and only one animal showed minor effects (which decreased at the 48-hour reading compared to the 24-hour reading).
Remarks	-

Section A6.1.5 Skin sensitisation

Annex Point IIA VI.6.1.5 6.1.5 Guinea Pig Maximisation Test

Table A6_1_5-1. Skin sensitisation scores - DCPP

Time point	DCPP-induced animals	Vehicle-induced animals
Skin reactions after challenge application of 5% DCPP in PEG 400 (left flank)		
24 h	1/10	0/5
48 h	1/10	0/5
Skin reactions after challenge application of PEG 400 (right flank)		
24 h	0/10	0/5
48 h	0/10	0/5

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

Official use only

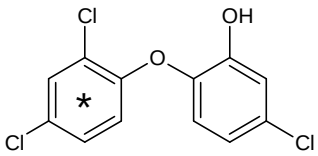
1 REFERENCE

1.1	**Reference**	[redacted](1994): ^{14}C-Triclosan: Absorption, Distribution, Metabolism and Elimination after Single/Repeated Oral and Intravenous Administration to Hamsters. [redacted] Project No. 351707, date: 1994-11-11, amended 1995-02-10 and 1995-08-25 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, – FDA Guideline for Metabolism Studies (1986) – OECD Guideline 417 (1984)
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Non-labelled parent compound**	
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	about 99%
3.1.2.1	Description	White powder
3.1.2.2	Purity	99.9%
3.1.2.3	Stability	Expiration date: December 1999 Storage stability: min. 2 years Stability in solvents: 48 h
3.2	**Labelled parent compound**	^{14}C-Triclosan
3.2.1	Lot/Batch number	0542
3.2.2	Specification	
3.2.2.1	Description	–
3.2.2.2	Purity	Radiochemical purity: 98.5 ± 1.0%
3.2.2.3	Stability	Verified
3.2.2.4	Radiolabelling	
3.3	**Test animals**	
3.3.1	Species	Syrian Golden Hamster

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

3.3.2	Strain	Bio-F1D, Alexander, hybrid	
3.3.3	Source	[redacted]	
3.3.4	Sex	♂ + ♀	
3.3.5	Age/weight at study initiation	Age: at least 11 weeks Body weight: 102-144 g	
3.3.6	Number of animals	118 males, 54 females	x
		Balance study (oral and i.v.): 4/sex/group; blood/plasma: 12/sex/group; bioretention after oral dosing: 16/sex/group	
		See Figure A6_2-1 for schematic overview on study design.	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Oral (gavage+feed), intravenous	
3.4.1	Application	**Gavage**	
3.4.1.1	Dosage	2 mg/kg bw (low dose) 200 mg/kg bw (high dose)	
3.4.1.2	Duration of treatment	single application	
3.4.1.3	Post-exposure period	168 h	
3.4.1.4	Specific activity of test substance	100 µCi/mg = 3.70 MBq/mg (low dose) 1 µCi/mg =0.037 MBq/ mg (high dose)	
3.4.1.5	Vehicle	10% ethanol, 20% cremophor, 70% aqueous CMC (1%) (v/v)	
3.4.1.6	Amount applied	7 mL/kg bw	
3.4.2	Application	**Feed**	
3.4.2.1	Dosage	2.2 mg/kg bw (low dose) 184 mg/kg bw (high dose)	
3.4.2.2	Duration of treatment	13 days	
3.4.2.3	Post-exposure period	168 h	
3.4.2.4	Specific activity of test substance	Unlabelled	
3.4.2.5	Vehicle	Diet	x
3.4.2.6	Amount applied	*Ad libitum*	
3.4.3	Application	**Intravenous**	
3.4.3.1	Dosage	2 mg/kg bw (low dose)	
3.4.3.2	Duration of treatment	Single injection	
3.4.3.3	Post-exposure period	168 h	
3.4.3.4	Specific activity of test substance	100 µCi/mg = 3.70 MBq/mg (low dose)	
3.4.3.5	Vehicle	Physiological saline	
3.4.3.6	Amount applied	1 mL/kg bw	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

3.5	**Examinations**		
3.5.1	Samples	Urine, faeces, cage wash, blood, bile, carcass, heart, lung, liver, stomach (contents were added to the intestinal tract), spleen, intestinal tract (with contents), adrenal glands, kidneys, gonads (ovaries and uterus or testicles arid epididymes), muscle, bones (femur), brain, skin (back region), fat and residual carcass.	
3.5.2	Sampling time (0 h = start of application)	Balance studies: Excreta: 24, 48, 72, 96, 120, 144, 168 h. Bile/plasma/organs: at sacrifice (168 h) Toxicokinetics: Blood: 0.5, 1, 2, 4, 8, 24, 48, 72, 96, 120, 144, 168 hours after the administration. An appropriate aliquot was separated, the remaining blood centrifuged and the plasma decanted. Organs/tissues/bile: At each time interval liver, kidney, muscle, fat and bile were sampled and stored at about -20 °C.	
		4 RESULTS AND DISCUSSION	
4.1	**Recovery of labelled compound**	Total recoveries of radioactivity ranged, on average, from 95.7 - 101.6% for males and females of the single dose groups and 98.2 - 102.8% of the repeated-dose groups.	
4.2	**Toxicokinetics**	Toxicokinetic parameters of ^{14}C-triclosan in hamsters are given in Table A6_2-1.	
4.3	**Absorption**	The results of plasma level studies and balance studies indicate that orally administered triclosan was rapidly and almost completely absorbed from the gastro-intestinal tract and efficiently eliminated mainly via the kidney into the urine as well as through biliary elimination into the faeces. The persistently higher plasma levels as compared to organs/tissues after single as well as repeated oral administration in both sexes indicate lack of accumulation of triclosan and/or its metabolites after repeated exposure to triclosan. Accordingly, radioactivity was highly extractable (81 - 95%) from liver and kidney.	x
4.4	**Distribution**	**Tissue Distribution after Single and Repeated Oral Administration at the Low Dose Level in Both Sexes** Single Oral Administration at the Low Dose Level. Males (Group 1) After low dose level administration in males, highest levels of radioactivity were found in the plasma (0.317 µg peq/g) and kidney (0.138 µg peq/g), followed by liver (0.068 µg peq/g), lung (0.091 µg peq/g) and intestinal tract (0.048 µg peq/g). Radioactivity levels in all other organs/tissues were below 40 µg peq/g, ranging from 0.005 - 0.036 µg peq/g. Repeated Oral Administration at the Low Dose Level. Males (Group 5) After repeated (13x) food supply at the low dose level in males again highest radioactivity levels were found in plasma (0.209 µg peq/g) followed by kidney (0.06 1 µg peq/g), liver (0.037 µg peq/g) and lung (0.062 µg peq/g). All other radioactivity levels were at or below 25 µg peq/g. As compared to single low dose administration in the male hamsters, radioactivity levels in organs/tissues were generally about 1.5 - 2 times lower after repeated (13x) food supply. Single and Repeated Oral Administration at the Low Dose Level. Females (Groups 3 and 7) After low dose level administration (single and repeated) in females,	

highest radioactivity levels were found in plasma (0.089 - 0.093 µg peq/g) followed by kidney (0.016 - 0.019 µg peq/g), liver (0.015- 0.017 µg peq/g) and lung (0.027 - 0.028 µg peq/g).
Hence, in the female hamsters, similar radioactivity levels in organs/tissues were found after single and repeated oral administration.

Tissue Distribution after Single and Repeated Oral Administration at the High Dose Level in Both Sexes

Single Oral Administration at the High Dose Level. Males (Group 2)
After single high dose level administration in males, highest radioactivity levels were found in plasma (16.175 µg peq/g) followed by kidney (3.254 µg peq/g), liver (3.433 µg peq/g) and lung (4.415 µg peq/g). Except for the intestinal tract after single high dose (2.361 µg peq/g), all other radioactivity levels were below 2 µg peq/g.
As compared to the single low dose level in males about 40-60 times higher radioactivity levels were found at about 100 times higher dose levels.

Repeated Oral Administration at the High Dose Level. Males (Group 6)
After repeated (13x) food supply at the high dose level in males again highest radioactivity levels were found in plasma (7.898 µg peq/g) followed by kidney (1.488 µg peq/g), liver (1.831 µg peq/g) and lung (2.204 µg peq/g). Radioactivity levels in all other selected organs/tissues were at or below 1.2 µg peq/g.
As compared to the single high dose administration in the male hamsters, radioactivity levels in organs/tissues were generally about 1.5-2 times lower after repeated (13x) food supply.

Single and Repeated Oral Administration at the High Dose Level. Females (Group 4 and 8)
After high dose level administration (single and repeated) in females, highest radioactivity levels were found in plasma (5.206 - 7.139 µg peq/g) followed by kidney (0.938 - 1.249 µg peq/g), liver (1.212 - 1.361 µg peq/g) and lung (1.470 - 1.915 µg peq/g). Except for carcass (1.1 - 1.6 µg peq/g), uterus after repeated high dose administration (1.565 µg peq/g) and ovaries after single high dose administration showing large variations (2.965 ± 1.349 µg peg/g), all remaining radioactivity levels were at or below 1.0 µg peq/g.
As compared to the low dose administration at 100 fold higher dose levels about 70-90 times higher radioactivity levels were found in organs/tissues of female hamsters.

After intravenous Administration without and with Repeated Oral Pre-treatment at the Low Dose Level

Single I.V. Administration at the Low Dose Level. Males (Group 9)
After single intravenous low dose administration in males, highest radioactivity levels were detected in plasma (0.421 µg peq/g) followed by the excretory organs kidney (0.148 µg peq/g) and liver (0.104 µg peq/g) as well as lung (0.125 µg peq/g). Except for the adrenal gland (0.056 µg peq/g), all remaining organs/tissues showed radioactivity levels at or below 0.050 µg peq/g.
As compared to the corresponding single oral balance group 1, similar radioactivity levels were found.

Repeated (13x) Food Supply Followed by Single I.V. Administration at the Low Dose Level. Males (Group 11)

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

At 168 hours, as compared to single intravenous administration very similar radioactivity levels were found in plasma (0.429 µg peq/g), kidney (0.146 µg peq/g), liver (0.096 µg peq/g), lung (0.131 µg peq/g), skin (0.061 µg peq/g), adrenal gland (0.064 µg peq/g) and remaining organs/tissues (below 0.050 µg peq/g).
Taking into account similar radioactivity levels in groups 1, 9 and 11, the corresponding repeated oral balance group 5 showed about 2 fold lower radioactivity levels.

Single I.V. Administration at the Low Dose Level and Repeated (13x) Oral Food Supply Followed by Single Intravenous Administration at the Low Dose Level. Females (Groups 10 and 12)
At 168 hours after low dose administration (single and repeated) in females, again highest radioactivity levels were found in plasma (0.046 - 0.055 µg peq/g). All radioactivity levels in organs/tissues were below 0.025 µg peq/g (single) and at or below 0.020 µg peq/g (repeated).
As compared to the corresponding oral balance groups 3 and 7 plasma radioactivity levels in the intravenous groups were 1.5 - 2 times lower as compared to the corresponding oral groups. In all organs/tissues very similar radioactivity levels were found.

4.5 Metabolism

See Figure A6_2-2 and Table A6_2-2.

Metabolism of Parent and Parent Conjugates

The major routes of triclosan metabolism are depicted in Figure A6_2-2. Besides the parent compound (M1), mainly two parent conjugates (parent sulphate M4 and parent glucuronide M7) were found. Additionally four non-parent conjugates (M5, M6. M8 and M9) were detected. All conjugates were susceptible to HCI-hydrolysis, resulting in free parent and mainly non-parent metabolite M2 which will be identified by GC-MS. The results at both dose levels indicate a less extensive phase I metabolism of triclosan in the hamster at the higher dose level of about 200 mg/kg against the low dose level of 2 mg/kg.

At Cmax (1-2 hours after administration) in male hamsters at both dose levels after single and repeated oral administration in plasma parent glucuronide and parent sulphate were found without detection of free parent. In kidney at Cmax mainly parent glucuronide as well as free parent and low to negligible amounts of parent sulphate were detected. The liver showed mainly parent as well as parent sulphate and low to negligible amounts of parent glucuronide.

In urine, except for group 3, free parent was only detected in small amounts (0.4 - 8.0% of the radioactivity recovered in the urine) and parent glucuronide represented the major urinary metabolite (55.6 - 86.7%). In faeces, mainly free parent was found and parent glucuronide occurred in small amounts. In both urine and faeces no parent sulphate was found.

As compared to the low dose levels at Cmax, at about 100 fold higher dose levels, the percentages of tree parent were similar in kidney (29 - 37%) and in liver (55 - 73 ¾). The percentage of parent conjugates were increased in plasma, liver and kidney: parent sulphate most pronouncedly increased in plasma (from 7-9% to 28-59%) and liver (from 8-16% to 36-38%). Parent glucuronide most pronouncedly increased after repeated high dose administration in plasma (from 24% to 56%) and kidney (from 48 to 60%), indicating in the hamster an enhanced conjugating capacity of triclosan at the high dose level of 200 mg/kg.

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

As compared to the low dose, parent sulphate concentrations were specifically enhanced (250-600 times) in the liver of the hamster at 100 times higher dose levels after single as well as repeated administration. Additionally, except for the enhanced parent glucuronide concentrations in the liver at the single high dose (300 times), all remaining parent and parent conjugates in liver and kidney showed at Cmax an about 100 told increase at about 100 times higher dose levels, indicating lack of saturation in conjugating capacity of triclosan in the liver and kidney of the hamsters at the dose levels tested.

The enhanced capacity of parent sulphate conjugation in the liver at the high dose level, and the occurrence of mainly parent glucuronide in kidney and urine as well as the lack of parent sulphate in urine, strongly suggests that the sulphate conjugate, produced in the liver and transported via the plasma to the kidney undergoes complete re-conjugation in the kidney to the glucuronide before being eliminated into the urine. Additionally, parent sulphate may be eliminated via the bile into the intestinal tract and is deconjugated by the intestinal microflora to free parent before being eliminated in the faeces.

Free parent and parent conjugates were rapidly eliminated from liver, kidney and plasma with half-lives less than 6 hours, indicating that elimination was highly efficient

Metabolism of Non-Parent Conjugates

At Cmax at the low dose levels after single and repeated oral administration, about 60-65% of the radioactivity recovered in plasma represented non-parent conjugates. In liver and kidney lower percentages (15 - 21%) of non-parent conjugates were found.

At Cmax at the high dose levels after single and repeated oral administration, only about 5-15% of the radioactivity recovered in the plasma represented non-parent conjugates. In liver negligible amounts (1-2%) and in kidney low amounts (3-10%) of non-parent conjugates were detected. Taking into account the increase in parent conjugates, these results indicate less extensive phase-I metabolism of triclosan at the high dose level of 200 mg/kg as compared to the low dose level of 2 mg/kg.

In representative urine pools at both dose levels (in general 0-96 hours), besides tree parent and parent glucuronide, tour non-parent conjugates accounted together for about 10 - 40% of the radioactivity recovered in the urine. All these conjugates were completely hydrolysed by HCI, mainly resulting in non-parent metabolite U2.

At later time points (1/4 and 1/8 Cmax) almost exclusively non-parent conjugates were detected in plasma, liver and kidney at low concentrations. Accordingly, longer half-lives (about 10-30 hours) were calculated for the non-parent conjugates as compared to free parent and parent conjugates (shorter than 6 hours) in plasma, liver and kidney. The plasma curve on total radioactivity represented therefore elimination of parent conjugates (α-phase) and subsequently elimination of non-parent conjugates (β-phase).

Finally as compared to the non-parent plasma concentrations at 48-56 hours (1/8 Cmax) after administration, about 6-7 times lower non-parent concentrations were found in the liver and about 2.5 times lower concentrations in the kidney. Accordingly, at sacrifice (168 hours), in the corresponding balance groups relative to plasma 4-5 fold lower amounts of total radioactivity were found in the excretory organs liver

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

		and kidney, indicating efficient elimination and lack of accumulation of non-parent conjugates of triclosan from organs/tissues of the hamster.	
4.6	**Excretion**	Single oral administration. After 168 hours, excretion of radioactivity via the urine ranged from 60.4 - 80.0% of the radioactivity administered in both sexes at both dose levels after single oral administration. Already 96 hours after the administration, 58.0 - 78.2% of the radioactivity administered was found in the urine. Accordingly, only small amounts of radioactivity (0.7 - 2.4%) were excreted via the urine during the time period from 96 to 168 hours. After 168 hours the amounts of radioactivity excreted via the faeces ranged from 12.9 - 28.9%. After single oral administration at the high dose level in both sexes only 12.9 - 15.0% were excreted via the faeces. As for urine, the major amount of the radioactivity was excreted within 96 hours. Taking into account the cage wash, total excreted radioactivity ranged from 94.9 - 100.5%. Repeated oral administration. After 168 hours excretion of radioactivity via the urine ranged from 63.7 - 70.0% of the radioactivity administered in both sexes at both dose levels after repeated oral administration Already 96 hours after the administration, 62.6 - 69.6% of the radioactivity administered was found in the urine. Accordingly, only small amounts of radioactivity (0.4 - 1 .1%) were excreted via the urine during the time period from 96 to 168 hours. After 168 hours the amounts of radioactivity excreted via the faeces ranged from 25.2 - 35.0%. After repeated oral administration at the high dose level in both sexes only 25.2 - 27.0% were excreted via the faeces. As for urine, the major amount of the radioactivity was excreted within 96 hours. Taking into account the cage wash, total excreted radioactivity ranged from 97.7 - 101.9%. Low amounts of radioactivity were found in plasma (0.1 - 0.6%) and residual carcass (0.3 - 0.8%) Negligible amounts (<0.05 - 0.1%) were found in organs/tissues and intestinal tract. Total recoveries of radioactivity ranged, on average, from 98.2 - 102.8% for males and females. In conclusion, the results of balance groups 1-8 indicated that absorbed radioactivity was efficiently eliminated mainly within 96 hours in both sexes at both dose levels. No differences were found between single and repeated oral administration.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	An ADME study with ^{14}C-triclosan was conducted in male and female Syrian hamsters. The study design is depicted in Figure A6_2-1. Low and high doses (2 and 200 mg/kg, respectively) were administered by gavage or by intravenous injection. The effect of repeated dosing was investigated by administration of 13 daily doses of unlabelled triclosan in feed. Radioactivity in excreta, tissues, and plasma was determined and the nature of the metabolites in these matrices was investigated by mass spectrometry.	x
5.2	**Results and**	Triclosan is rapidly absorbed (via the oral and i.v. routes of administration) in the hamster. There is no indication for	x

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

	discussion	bioaccumulation/bioretention of triclosan following repeated oral doses. Triclosan is widely distributed in organs and tissues, with well-perfused, and excretory, organs such as liver and kidney, as well as lung, heart, GI tract, and gall bladder showing highest levels. Following absorption, the parent triclosan compound was found to be metabolised to both glucuronide and sulphate conjugates. The predominant excretion route was via the urine. The majority of the applied dose was excreted via urine within 96 h post dose. Excretion via urine and faeces after 168 h post dose was complete. There is no evidence for enterohepatic circulation.
5.3	**Conclusion**	Triclosan is efficiently absorbed after oral dosing. Excretion takes place via urine as glucuronide and sulphate conjugates.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

Evaluation by Competent Authorities

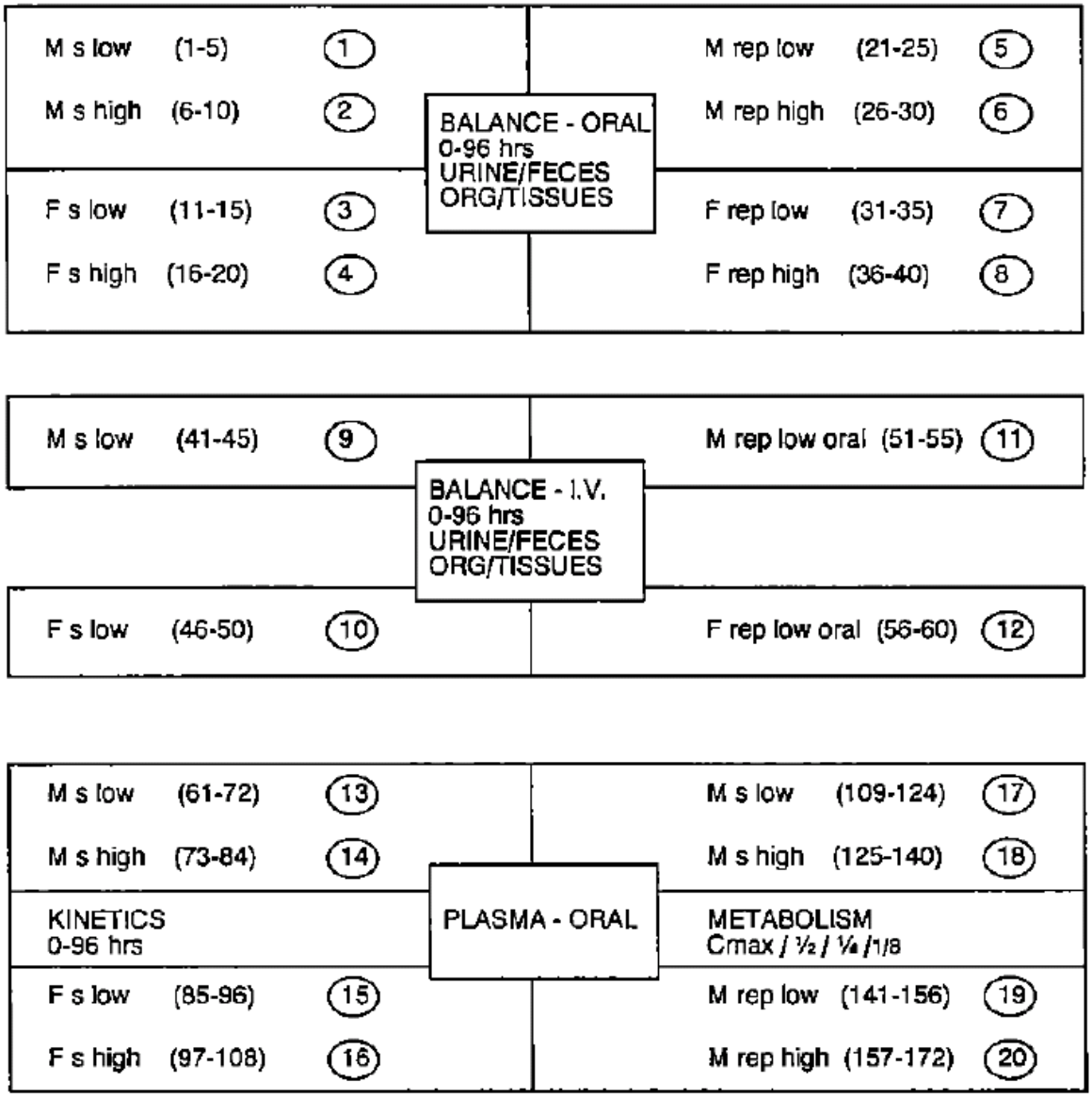
EVALUATION BY RAPPORTEUR MEMBER STATE

Date	March 2010
Materials and Methods	3.3.6: *Amendment:* In the first experiment a single oral dose via gavage was administered at doses of 2.0 mg/kg in both ♂ (group 1) and ♀ (group 3) or approx. 200 mg/kg in ♂ (group 2)/ ♀ (group 4). Secondly, animals were administered 2 or 200 mg/kg unlabeled triclosan via the diet for 13 days, then dosed with a single oral dose of radioactive labelled triclosan via gavage (2.0 mg/kg in ♂ (group 5)/ ♀ (group 7) or 200 and 203 mg/kg in ♂ (group 6)/ ♀ (group 8) or intravenous (2.0 mg/kg in ♂ (group 11) and ♀ (group 12). A third experiment was performed with a single 2.0 mg/kg ^{14}C-triclosan intravenous dose with both ♂ (group 9) and ♀ (group 10). Blood/plasma level studies were performed on 12 hamsters/sex/dose at dose levels of 2.0 and 200 mg/kg in ♂ (group 13)/♀ (group 15) and ♂ (group 14)/♀ (group 16). Bioretention and metabolite distribution experiments were performed on 16 hamster males/dose with doses of 2.0 or 200 mg/kg of via gavage (groups 17 and 18, respectively) and on 16 hamster males/dose fed unlabeled triclosan for 13 days before receiving gavage doses of 2.0 or 200 mg/kg of ^{14}C-triclosan (groups 19 and 20). 3.4.2.5: *Addition:* Vehicle: unlabeled triclosan was dissolved in 1 L Acetone and mixed with 10 kg diet.
Results and discussion	*4.3: Addition:* At least 68/81% of the low dose and 89/84% of the high dose were absorbed from the gastrointestinal tract into systemic circulation, for males/females respectively. *5.1:* Only fraction U2 was investigated by GC/MS. U2 was identified as 2,4,4-trichloro-2,5,dihydroxydiphenylether in addition to HCl- or enzymatic treatment. *Amendment:* Following absorption, the parent triclosan compound was found to be metabolised to <u>at least 7 metabolic fractions</u> including glucuronide and sulphate conjugates. <u>At the high dose level parent sulphate in plasma and liver (in conjunction with decreased glucuronidation) of hamster is increased. Elimination from tissues (plasma, liver, kidney) to 1/8 Cmax was calculated between 48-56 hours.</u>
Conclusion	Agreed with the additions above.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

Figure A6_2-1 **Schematic overview of ADME study design with ^{14}C-Triclosan in hamsters**



M = male / F = female / s = single / rep = repeated (13x) food supply
( ) = animal numbers

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(01) ADME of triclosan in Syrian hamsters

Table A6_2-1: Plasma toxicokinetics of ^{14}C-triclosan after single oral administration at two dose levels to male and female hamsters

Kinetic Parameter*	Dose Level [mg/kg]			
	2.0 (single)	199 (single)	2.0 (single)	201 (single)
	Group No.			
	13 ♂	14 ♂	15 ♀	16 ♀
C_{max} [µg/g]	7.684	359.171	7.357	384.920
T_{max} [h]	1	1	1	1
$T_{1/2}$ [h]	29.1	32.0	24.5	27.0
AUC [(mg/L)×h]	178.0	6010	79.8	4298
$AUC_{high\ dose}$ / $AUC_{low\ dose}$	1	34	1	55
Time interval after regression [h]	1-168	1-168	1-168	1-168
Regression coefficient R	0.966	0.927	0.928	0.908
k_1 [h]	-0.024	-0.022	-0.028	-0.026
C_0 [mg/L]	5.02	164.4	2.86	140.5
C_{168}** [mg/L]	0.13	7.26	0.06	3.79

* Based on $ln\ C_t = ln\ C_0 - k_1 \times t$ (first order elimination kinetics)
** Sacrifice time

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of triclosan in Syrian hamsters

Table A6_2-2: Comparison of urinary (U) and faecal (F) metabolite patterns on TLC after oral and i.v. administration of ^{14}C-triclosan

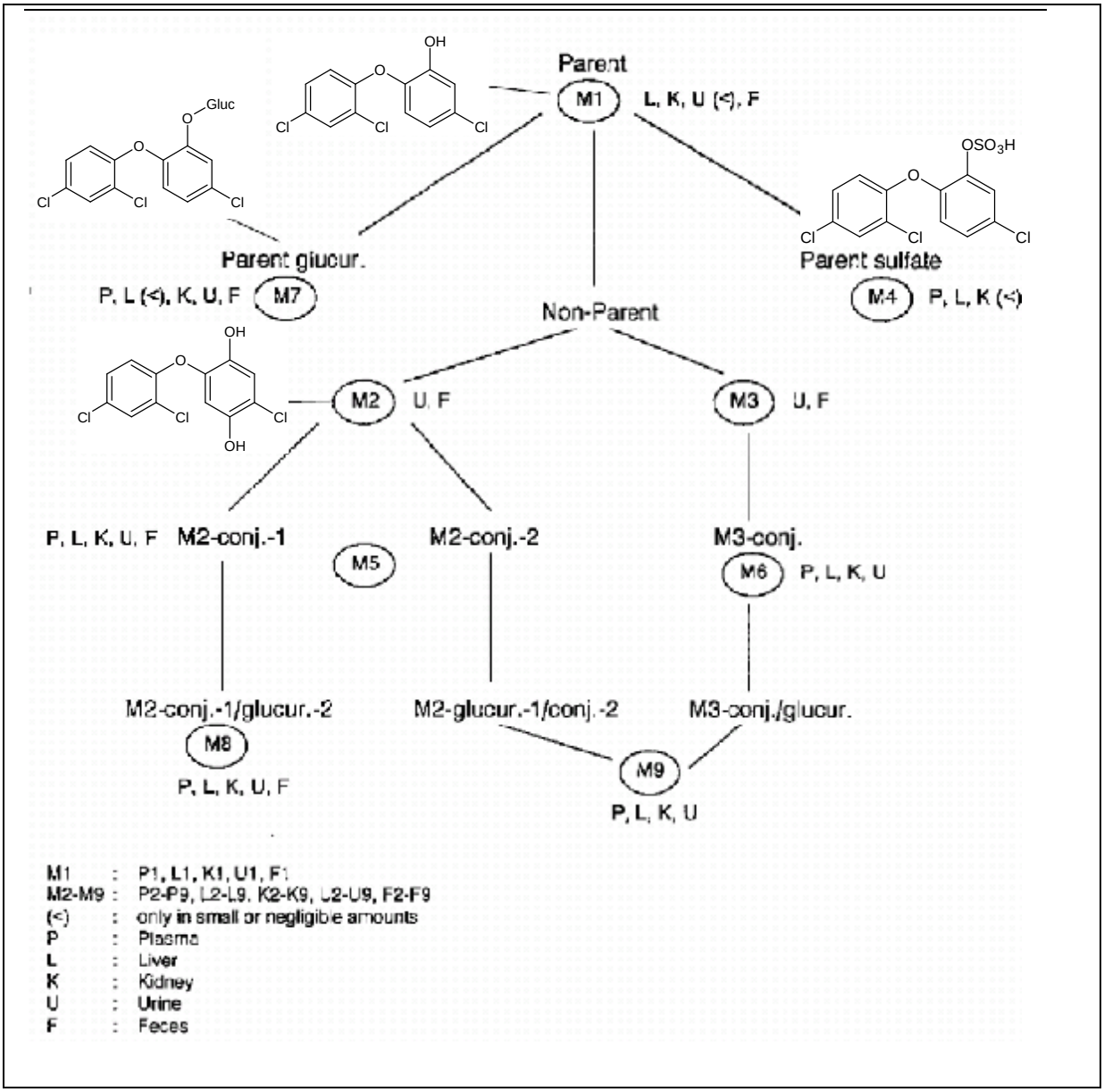
Metabolite Code	Identity	Dose Level (mg/kg)							
		2.0 (S)	213 (S)	2.0 (S)	203 (S)	2.0 (R)	200 (R)	2.0 (R)	200 (R)
		Group - No.							
		1 (M)	2 (M)	3 (F)	4 (F)	5 (M)	6 (M)	7 (F)	8 (F)
Urine:									
U1	Parent	1.4	5.4	33.1	6.3	0.7	0.9	0.6	0.2
U2	Unknown*	---	---	2.1	0.6	---	---	---	---
U3	Unknown	---	---	2.8	---	0.4	---	1.1	---
U5	U2-conj.	1.6	1.5	12.2	0.5	0.8	0.4	1.5	0.6
U6	U3-conj.	5.7	4.3	4.4	3.4	4.2	3.8	5.4	4.0
U7	Parent glucur.	32.2	55.6	9.5	60.2	37.8	57.1	42.6	59.2
U8	U2-conj.	11.5	7.2	9.5	4.6	14.0	4.7	10.4	3.3
U9	U2/U3-conj.	5.6	4.2	2.8	2.7	4.7	2.4	3.0	0.9
Subtotal		58.0	78.2	76.4	78.3	62.6	69.3	64.6	68.2
Feces:									
F1	Parent	13.8	9.8	6.9	11.4	19.0	21.1	16.8	19.0
F2	(U2)	6.8	1.3	2.5	0.6	7.1	1.0	3.8	0.4
F3	(U3)	1.3	---	0.6	---	0.7	---	0.9	---
F5	(U5)	0.9	---	0.8	---	0.6	0.6	2.2	---
F7	Parent glucur.	1.8	0.2	1.3	1.2	3.6	0.4	5.1	4.8
F8	(U8)	0.6	---	0.3	---	---	---	0.7	---
Subtotal		25.2	11.3	12.4	13.2	31.0	23.1	29.5	24.2
TOTAL		83.2	89.5	88.8	91.5	93.6	92.4	94.1	92.4

* Metabolite "U2" was later identified (study amendment) as a hydroxy-metabolite of the parent compound (see Figure A6_2-2)

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of triclosan in Syrian hamsters

Figure A6_2-2: Major routes of metabolism of triclosan in hamster



Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(03) Oral toxicokinetics in human volunteers

			Official use only
		1 REFERENCE	
1.1	**Reference**	Sandborgh-Englund, G. *et al.* (2006): Pharmacokinetics of Triclosan Following Oral Ingestion in Humans. Institute of Odontology, Karolinska Institutet, Huddinge, Sweden *Journal of Toxicology and Environmental Health, Part A*, 69:1861–1873 (published).	
1.2	**Data protection**	No	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
2.2	**GLP**	No	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test compound**	Triclosan, a gift from Ciba-Geigy	x
3.1.1	Lot/Batch number	Not reported	
3.1.2	Specification	Not reported	
3.2	**Test subjects**		
3.2.1	Species	Human	
3.2.2	Source	Volunteers	
3.2.3	Sex	♂ + ♀	
3.2.4	Age/weight at study initiation	♂: median age: 29 yr (range 26-42), median bw: 77 kg (range 76-95) ♀: median age: 27 yr (range 26-30), median bw: 57 kg (range 51-77)	
3.2.5	Number of subjects	5 males, 5 females	
3.2.6	Controls	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Application	**Drink**	
3.3.1.1	Dosage	median: 58 µg/kg bw; 95%-CI: 49-67 µg/kg bw	
3.3.1.2	Duration of treatment	single application	
3.3.1.3	Post-exposure period	196 h	
3.3.1.4	Vehicle	Mouthwash solution (Colgate Total)	
3.3.1.5	Amount applied	4 mg	x
3.4	**Examinations**		
3.4.1	Samples	Urine, blood	
3.4.2	Sampling time (0 h = start of application)	After exposure, blood samples were drawn after 1, 2, 3, 4, 6, 9, 24, 48, 72, 96, and 192 h. Baseline urinary excretion of triclosan was established from 24-h urine collections at 6 and 3 d prior to exposure. After exposure, 24-h urine collection was performed from 0 to 24 h, 24 to 48 h, 48 to 72 h, 72 to 96 h, and 172 to 196 h.	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(03) Oral toxicokinetics in human volunteers

4 RESULTS AND DISCUSSION

4.1 Recovery of labelled compound

Not determined

4.2 Toxicokinetics

Toxicokinetic parameters of ^{14}C-triclosan in humans are given in Table A6_2-1.

4.3 Absorption

The absorption phase of triclosan was very fast, with Cmax being reached within 3 h after exposure, followed by a bi-exponential decline. Several attempts were made using two-compartment models with absorption phase and a one-compartment model with absorption phase. However, an acceptable fit could only be achieved using the intravenous (IV) bolus, two-compartment model.

4.4 Excretion

The terminal half-life of triclosan in plasma was 19 h (median). Plasma clearance (relative) was calculated to be 0.041 ± 0.008 L/h/kg body weight (mean ± 95% confidence interval). In Figure A6_2-1, typical curves of the time course of triclosan in plasma are shown. One of the subjects (number 7) had by mistake used triclosan-containing toothpaste 6 d after exposure, and the plasma level at the final sampling occasion is clearly elevated.

The unconjugated fraction of triclosan in plasma increased parallel to the total level as determined during the first 6 h after exposure (Table A6_2-2). At other times, the concentration of unconjugated triclosan was below the limit of detection.

The urinary excretion increased after exposure, and the major fraction was excreted within the first 24 h. Between 24 and 83% (median 54%) of the oral dose was excreted within the first 4 d after exposure (Figure A6_2-2). The correlation between urine and plasma data was high. The excretion of triclosan approached baseline levels within 8 d after exposure.

Urinary excretion half-life was calculated to be 11 h (median, range 7–17). The relative renal clearance constituted 57 ± 12% (mean ± 95% confidence interval) of the total.

There was a significant correlation between plasma clearance and relative renal clearance, as well as an inverse correlation between the level of unconjugated triclosan in plasma and relative renal clearance. The amount of unconjugated triclosan in urine was low (Table A6_2-2).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Ten subjects (5 males and 5 females, median age 28 yr) took part in the study. They were judged to be healthy by medical examination and standard blood chemistry.

All personal hygiene products (toothpaste and other oral health care products, deodorant soaps, underarm deodorants, shower gels, shampoo, face wash) were monitored for triclosan content (from product content labelling).

The subjects were monitored for triclosan exposure and also were interviewed regarding hygiene habits, that is, use of personal hygiene products. Five of them used triclosan-containing products: in four cases triclosan toothpaste, one of them both triclosan toothpaste and triclosan mouthwash, and in one case face wash only. The participants were asked to cease using triclosan-containing products from the first

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(03) Oral toxicokinetics in human volunteers

		sampling occasion, 6 d prior to the experimental exposure.	
		All subjects received non-triclosan toothpaste, which they were instructed to use during the washout and sampling period. Pre-exposure monitoring of triclosan in plasma and urine was performed in order to ensure that the concentrations were low at the time of exposure. The subjects were asked about any adverse effect and compliance in conjunction to each sampling occasion.	
		Baseline levels of triclosan in plasma were determined from blood samples collected 6 and 3 d prior to exposure, and on the morning of the experimental day (d 0). After exposure, blood samples were drawn after 1, 2, 3, 4, 6, 9, 24, 48, 72, 96, and 192 h. Baseline urinary excretion of triclosan was established from 24-h urine collections at 6 and 3 d prior to exposure. After exposure, 24-h urine collection was performed from 0 to 24 h, 24 to 48 h, 48 to 72 h, 72 to 96 h, and 172 to 196 h.	
5.2	**Results and discussion**	Triclosan levels in plasma increased rapidly, with a maximum concentration within 1 to 3 h, and the terminal plasma half-life was 21 h. The major fraction was excreted within the first 24 h. The accumulated urinary excretion varied between the subjects, with 24 to 83% of the oral dose being excreted during the first 4 d after exposure.	x x
		In males, the mean relative urinary clearance was 0.037 L/h/kg bw (95% CI: 0.027-0.047). In females, mean plasma clearance was 0.044 L/h/kg bw (95% CI: 0.036-0.053).	x
		The plasma clearance (oral dose/plasma AUC) in males and females is 0.020 and 0.024 L/h/kg bw, respectively.	
		Thus, there is no marked sex difference regarding elimination of triclosan from plasma.	x
5.3	**Conclusion**	Triclosan appears to be readily absorbed from the gastrointestinal tract and has a rapid turnover in humans.	x
5.3.1	Reliability	2	
5.3.2	Deficiencies	The study has reporting deficits which are normal for published work.	x

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(03) Oral toxicokinetics in human volunteers

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2010
Materials and Methods	3.1: *Replace:* 13 ml of a mouth wash solution containing 0.03% triclosan (no further information concerning the identity of the a.i. stated) 3.3.1.5: *Amend:* corresponds to 0.04-0.08 mg/kg/bw
Results and discussion	5.2: Triclosan levels in plasma increased rapidly, with a maximum concentration within 1 to 3 h, and the terminal plasma half-life was 19 h (median). According to the authors the relatively large difference in accumulated urinary excretion may be due to individual variation in bioavailability, variation in distribution kinetics and variation in renal clearance. The inverse correlation between unconjugated tricolsan in plasma and renal clearance might imply that individual differences. *Replace:* <u>The mean renal clearance was 0.041 L/h/kg.</u> *Reason:* Only the renal clearance for both genders was calculated in the study. The calculation of the plasma clearance is not feasible based on findings from the author that the elimination process does not follow linear elimination kinetics. Also for this kind of calculation of the clearance the dose injected intravenously (and not the oral dose) should be used.
Conclusion	5.3: *Addition:* The author also pointed out that baseline concentration complicated the interpretation of the results. The large variation in cumulative urinary excretion after 4 days such a general statement that tricolsan has a rapid turnover in humans is not fully substantiated by the presented data. 5.3.2: *Addition:* No primary data and calculations were reported.
Reliability	2
Acceptability	Acceptable
Remarks	-

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2(03) Oral toxicokinetics in human volunteers

Table A6_2-1: Pharmacokinetic Parameters of Triclosan in Plasma Following a Single Oral Dose of 4 mg Triclosan

Subject	Dose (µg/kg)	C_{max}[a] (µg/L)	T_{max}[b] (h)	AUC[c] (h•µg/L)	β Half-life[d] (hr)	Cl/F[e] (L/h/kg)
1	58	148	2.0	1.8	43	0.056
2	78	318	1.0	4.6	29	0.029
3	53	186	1.0	1.7	12	0.056
4	74	211	2.0	2.4	13	0.054
5	42	165	2.0	2.2	20	0.033
6	52	161	1.0	2.4	10	0.038
7	70	354	1.0	2.7	19	0.045
8	50	217	1.0	2.0	—[f]	0.043
9	51	228	1.0	3.4	15	0.026
10	51	195	3.1	3.3	29	0.027
Mean	58	218	1.5	2.6	21	0.041
95% CI	49–67	170–267	1.0–2.0	2.0–3.3	13–29	0.032–0.049

Note. C_{max} and T_{max} are direct observations. AUC, β half-life, and Cl/F originate from the pharmacokinetic modeling.

[a]The maximum triclosan concentration in plasma after exposure (net level).

[b]Time after start of exposure when C_{max} is reached.

[c]Area under the concentration-time curve from time 0 extrapolated to infinity.

[d]Terminal half-life of triclosan in plasma.

[e]Clearance/fraction of dose absorbed per kg body weight.

[f]—, Not stated due to unacceptable CV% in model

Table A6_2-2: Percent Unconjugated Triclosan of Total in Plasma and Urine at Different Times Following a Single Oral Dose Exposure of 4 mg Triclosan (Mean and 95% Confidence Interval)

	Plasma, time after exposure					Urine, time after exposure	
	1 h	2 h	3 h	4 h	6 h	0–24 h	24–48 h
Mean	27	32	33	34	38	0.5	0.9
95% CI	22–33	24–40	26–40	28–40	29–47	0.3–0.6	0.6–1.2

Figure A6_2-1: Change in plasma triclosan concentration (net concentration) in 10 subjects after ingestion of 4 mg triclosan in a mouthwash solution at d 0.
For 8 subjects, the plasma levels at d 8 were equal to or below baseline levels, which means that they are not shown in the graph. One subject had used triclosan-containing toothpaste 6 d after exposure; the plasma level at the final sampling occasion is notably elevated.

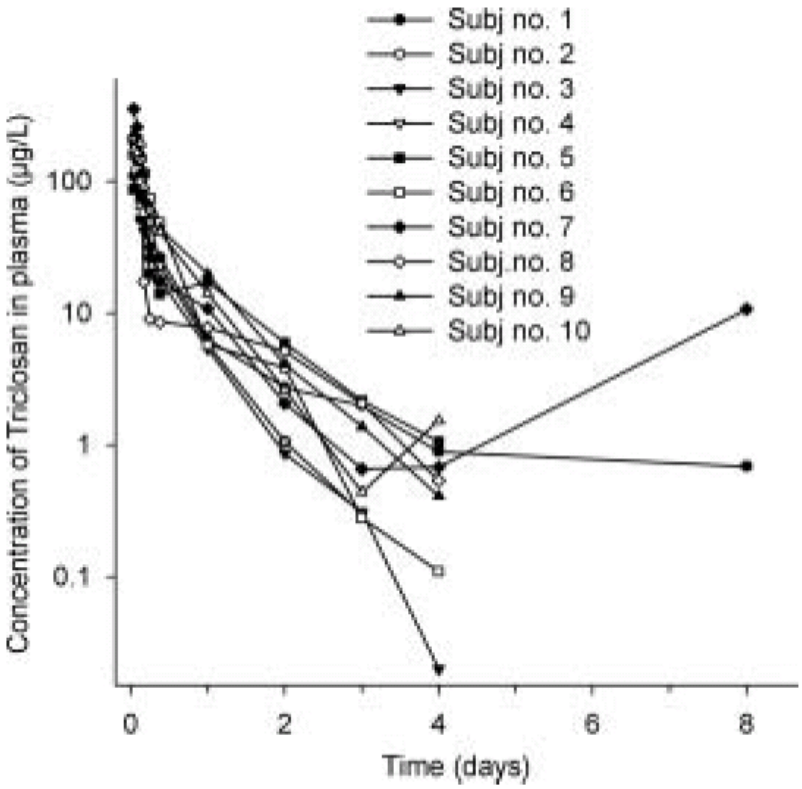
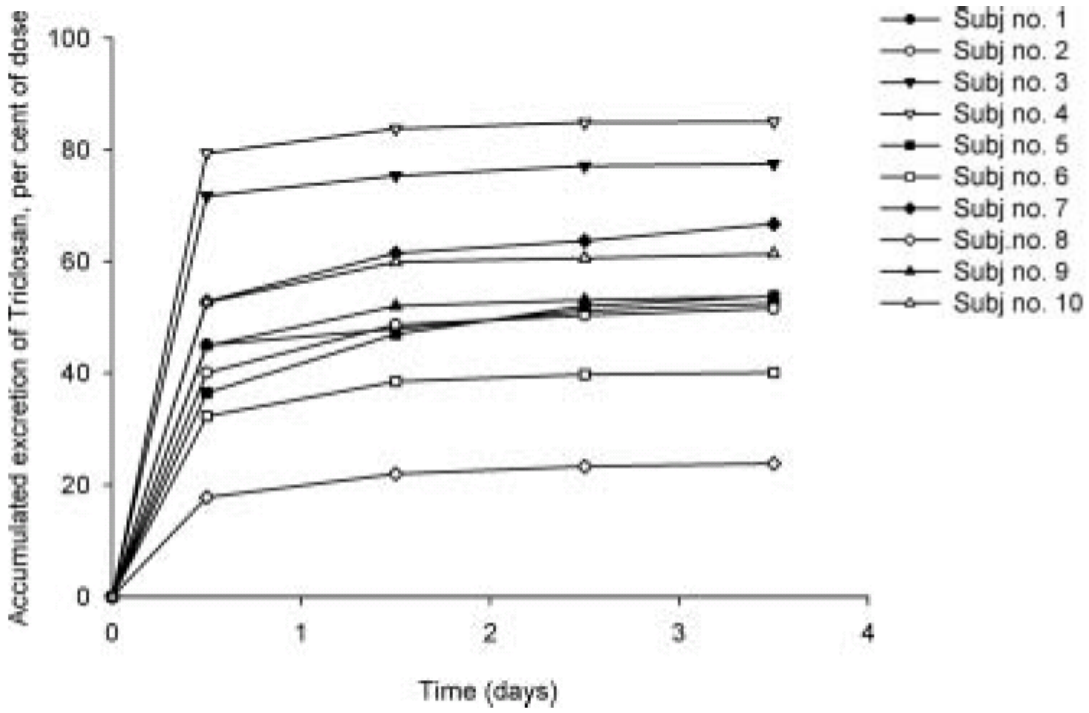


Figure A6_2-2: Accumulated excretion of triclosan in urine in 10 subjects (% of dose) receiving an oral dose of 4 mg triclosan at d 0.



Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Official use only

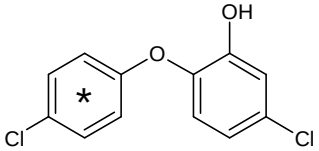
1 REFERENCE

1.1	**Reference (1)**	[redacted](2008a): Absorption, Distribution, Excretion and Metabolism of ^{14}C-DCPP in the Hamster After Oral Administration. [redacted] Project No. B42276, date: 2008-10-30, (unpublished).
1.2	**Reference (2)**	[redacted](2008b): Disposition of ^{14}C-DCPP in the Hamster After Multiple Oral Administrations. [redacted] Project No. B42287, date: 2008-10-30, (unpublished).
1.3	**Data protection**	Yes
1.3.1	Data owner	BASF SE
1.3.2	Companies with letter of access	–
1.3.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline 417 (1984)
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Non-labelled parent compound**	
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Beige solid
3.1.2.2	Purity	99.94%
3.1.2.3	Stability	Expiration date: March 31, 2009 The stability of the test item in the administration vehicle was checked by HPLC at the time of the application separately for each dosing group.
3.2	**Labelled parent compound**	^{14}C-DCPP
3.2.1	Lot/Batch number	[redacted]
3.2.2	Specification	
3.2.2.1	Description	–
3.2.2.2	Purity	Radiochemical purity: 99.3%
3.2.2.3	Stability	The radiochemical purity will be checked at the time point of administration. Therefore no expiration date is needed.
3.2.2.4	Radiolabelling	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

3.3	**Test animals**		
3.3.1	Species	Syrian Golden Hamster	
3.3.2	Strain	LAK:LVG(SYR) BR	x
3.3.3	Source	[redacted]	
3.3.4	Sex	Single dose: ♂ + ♀ Repeated dose: ♂	
3.3.5	Age/weight at study initiation	Age: not reported Body weight: 100 g and 80 g for males and females (single dose), 180 g (males, repeated dose)	x
3.3.6	Number of animals	Single dose: 17 ♂ + 8 ♀ Repeated dose: 7 ♂	
3.3.7	Control animals	No	
3.4	**Administration/ Exposure**	Oral gavage	
3.4.1	Application	**Gavage**	
3.4.1.1	Dosage	2 mg/kg bw (single low dose) 200 mg/kg bw (single high dose) 2 mg/kg bw (repeated low dose)	
3.4.1.2	Duration of treatment	single application / 14 applications (all with labelled material)	x
3.4.1.3	Post-exposure period	48 h (single dose)/168 h (repeated dose)	x
3.4.1.4	Specific activity of test substance	Undiluted radiolabelled material: 4.37 GBq/mmol (118 mCi/mmol) ≅ 16.9 MBq/mg (456 µCi/mg) Application solutions: Single low dose, mass balance studies: 7244 kBq/mg Single high dose, mass balance studies: 289 kBq/mg Single low dose, toxicokinetics: 778 kBq/mg Repeated low dose: 3340 kBq/mg.	
3.4.1.5	Vehicle	PEG 300 / water (70:30, v:v)	
3.4.1.6	Amount applied	Single doses: 6 mL/kg bw Repeated doses: 3 mL/kg bw	
3.5	**Examinations**		
3.5.1	Samples	Single doses: Urine, faeces, cage wash, blood/plasma, bile, carcass, adrenals, bile fluid, blood, brain, carcass, epididymis, fat (white), femur, heart, kidneys, liver, lungs, muscle, ovaries, pancreas, skin, spleen, testis, thymus, uterus Repeated doses: Urine, faeces, cage wash, blood/plasma	x
3.5.2	Sampling time (0 h = start of application)	Single doses: Urine: 0-24, 24-48, 48–72, and 72-96 hours after administration. Faeces: 0-24, 24-48, 48–72, and 72-96 hours after administration Necropsy: at the end of the experiment, i.e. 96 hours after administration Repeated doses: Urine was collected only from Animal 6 and 7 in daily intervals.	x

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

		Faeces were collected only from Animal 6 and 7 in daily intervals. Blood was sampled immediately before sacrifice: Animal 1 = 24 hours after 1st dose Animal 2 = 24 hours after 4th dose Animal 3 = 24 hours after 7th dose Animal 4 = 24 hours after 10th dose Animal 5 = 24 hours after the last dose Animal 6 = 72 hours after the last dose Animal 7 = 168 hours after the last dose	
		4 RESULTS AND DISCUSSION	
4.1	**Recovery of labelled compound**	Single dose: (see Table A6_2-1) low dose: 84.01-86.49% high dose: 91.82-94.22%	x
		Repeated dose: 97.19%	
4.2	**Toxicokinetics**	Single dose: The maximum concentration level in blood and plasma was achieved 4 hour after administration, accounting for 1.561 and 3.365 ppm DCPP equivalents, respectively. Thereafter the concentration decreased with an initial half-life (4-24 h) of about 6 hours. A second slower elimination phase was observed between 24 and 96 hours after administration. The terminal half lives (24-96 h) accounted for 26 and 36 hours for blood and plasma, respectively. The AUC values (0-96 h), being an index of bioavailability, were calculated to be 26.8 and 53.4 µg×h/g for blood and plasma, respectively. The radioactivity in blood was located predominately in plasma as indicated by the AUC blood/plasma ratio of 1:2 (see Figure A6_2-1).	
		Repeated dose: The trough concentrations in blood, i.e. 24 hours after last dosing, did not increase with ongoing dosing. The plateau of blood concentration was reached just after the first administration at a level of about 0.3 ppm DCPP equivalents. After the last of 14 consecutive daily doses the blood concentration declined rapidly reaching half the maximum concentration within 24 hours.	x
		The blood/plasma ratio was calculated to be 0.5, indicating that the radioactivity found in whole blood was located predominantly in the plasma (see Figure A6_2-2).	
4.3	**Absorption**	Single dose: After oral administration at the low dose level the radioactivity was rapidly and almost completely absorbed from the gastrointestinal tract into system circulation. The maximum concentration level in blood and plasma was achieved 4 hour after administration.	
		The amount absorbed was calculated based on the radioactivity determined in urine, cage wash, and residues in tissues and carcass. At least 81/79% of the low dose and 89/79% of the high dose were absorbed from the gastrointestinal tract into systemic circulation, for males/females respectively. The actual extent of absorption may be even higher since the total recovery of the low dose level amounted only for 86% (males) and 84% (females), which is caused by a loss radioactivity in the wire grid of the metabolism cages.	x
		Repeated dose: The orally administered DCPP was almost completely absorbed from the gastro- intestinal tract into the systemic circulation.	
4.4	**Distribution**	Single dose: (see Table A6_2-2) For the low dose level, the concentration in tissues and organs were generally low at 96 hours after administration. However all selected tissues and organs showed	

concentration levels above the LOQ level except in brain (females). Beside the bile fluid the highest concentrations were found in plasma, kidneys liver, and lungs. Lowest concentration was found in brain. Females revealed significantly lower residue levels as compared to males.

At the high dose level the concentration in tissues and organs were correspondingly higher. Again the highest concentrations were found in bile fluid and plasma, followed by adrenals, kidney, liver, and lung. Also at the high dose level the lowest concentration was found in the brain with level very close to LOQ, indication that DCPP and/or its radiolabelled metabolites are not able to pass the blood/brain barrier. At the high dose level the concentration levels in males and females were comparable.

Repeated dose: (see Table A6_2-3) The trough concentration in tissues and organs showed an almost constant level for all of the selected tissues and organs during the dosing period. The steady state was reached just at the first sampling time point at day 2. The bile bladder revealed a high concentration of radioactivity, i.e. 0.771 ppm at Day 2, indication a biliary excretion of the absorbed DCPP.

The high concentration of radioactivity in the bile fluid caused a high range of variation of the concentration values of the bile bladder. The bile bladder could not be assigned in the whole body sections of animal 3 and 4.

The highest steady state levels were found in blood, kidney cortex and lungs, accounting for 0.305 ppm, 0.224 ppm, and 0.221 ppm DCPP equivalents, respectively. All other tissues and organs showed plateau levels below 0.200 ppm. Lowest plateau levels were found in muscle, thymus, and bone, not exceeding 0.030 ppm. In brain tissue all measured concentrations were below LOQ, indicating that DCPP and/or its radiolabelled metabolites are not able to pass the blood brain barrier.

After the last of 14 consecutive daily doses the concentration levels in tissues and organs decreased with terminal half lives (Day 15-21) of 31 to 54 hours. Within seven day after the last dosing all selected tissues and organs reached concentration levels below LOQ except for liver, kidney, and bile bladder.

4.5 Metabolism

Single dose: (see Table A6_2-4)
Urine: For the low dose level (Group 1) the chromatography revealed a metabolite pattern, consisting of at least 16 metabolite fractions. The urinary metabolite pattern was dominated by fraction U11 (DCPP-glucuronide conjugate) and U7 (glucuronide and sulphate conjugates of hydroxylated DCPP) accounting for 17.6/21.7% of the dose and 12.4/10.0% of the dose for males/females, respectively. Only low amount in urine was found as unchanged parent, i.e. 6.7/3.3% of the dose. The urinary metabolite pattern was qualitative and quantitative very similar for males and females.

For the high dose level the urinary metabolite pattern was comparable to that observed at the low dose level. Again the urinary metabolite pattern was dominated by fraction U11 (DCPP glucuronide conjugate) followed by U7 (glucuronide and sulphate conjugates of hydroxylated DCPP), U13 (Hydroxy -DCPP), and low amounts of U15 (DCPP-sulphate conjugate).

Unchanged parent was found at a level of 2.0/2.5% of the dose for males/females, respectively. All other metabolite fractions were minor

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

not exceeding 2% of the dose.

Also at the high dose level metabolite pattern was qualitative very similar for males and females. The apparent differences in quantity are caused by the lower amount of radioactivity found in the urine of females. This lower amount radioactivity in the urine was found at the end of the experiment in the cage wash specimen.

The structures of the identified metabolites are summarised in Table A6_2-6.

Faeces: HPLC analysis of faecal extracts revealed at least 8 metabolite fractions. The extractability and the metabolite pattern of the faeces were essentially identical for both sexes and both dose levels with slight quantitative variations. These quantitative variations are mainly based on the variation in faecal excretion. Unchanged parent (F5) was excreted via faeces at a rate of 2-3% of the low dose and 4-8% of the high dose. Besides parent only minor fractions were found not exceeding 1% of the dose (see Table A6_2-7).

Repeated dose:
Urine: The chromatography revealed a metabolite pattern, consisting of at least 16 metabolite fractions. The pattern was qualitatively and quantitatively not influenced by the multiple dosing. The urinary metabolite pattern was dominated by fraction U11 (DCPP-glucuronide conjugate) and U7 (DCPP hydroxy-glucuronide conjugate) accounting for 40-42% of the dose and 12-17% of the dose (see Table A6_2-5).

Unchanged parent was found only in the range of 0.9-2.5% of the dose.

A somewhat unexpected metabolite pattern was found for the pool G1-U3m (Day 14-15). The high amount of unchanged parent is attributed to a contamination of the urine with faecal metabolites. It is assumed that the dirt in the funnel with attached faeces and diet was rinsed by the excreted urine, which is an explanation for the high concentration of unchanged parent in this specimen. The additionally analyzed pool G1-U4m (Day 13-14) showed a comparable metabolite pattern to the other analyzed time intervals. Thus the outcome of the analyzed G1-U3m has to be considered as artefact.

Faeces: (see Table A6_2-8) HPLC analysis of faecal extracts revealed at least 8 metabolite fractions. The extractability and the metabolite pattern of the faeces were essentially identical for all three selected sampling intervals with slight quantitative variations. These quantitative variations are based on the time shift of faecal excretion for the first time interval after start of dosing. Unchanged parent (F6) was excreted via faeces at a rate of 8-15% of the daily dose. Besides parent only minor fractions were found not exceeding 4% of the daily dose. One unknown metabolite fraction was observed especially in the later time intervals (day 7-8 and 14-15) accounting for about 2% of the daily dose. Since the retention time of this unknown faecal metabolite fraction corresponds with the major metabolite fraction of the urine, it is assumed that this fraction is caused by a cross contamination with excreted urine.

Proposed metabolic pathway: see Table A6_2-9

4.6 Excretion

Single dose: The absorbed radioactivity was predominately excreted with the urine, accounting for 80/78% of the low dose and 88/78% of the high dose for males/females, respectively. Significantly lower amounts were excreted with the faeces, accounting for 5.5/4.6% of the low dose and 5.3/12.6% of the high dose. Ninety-six hours after

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

		administration less than 2% of dose remained in the animals for both dose levels. Repeated dose: During the dosing period, i.e. 14 consecutive daily doses, a steady state in terms of excretion was reached just 1 day after the first administration. The amount of daily excretion remained nearly constant until the end of dosing, accounting for about 78% and 16% of the daily dose for urine and faeces, respectively. Due to the almost constant excretion profile the systemic absorption seems to be uninfluenced by the multiple-dose regimen.	x
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The fate of DCPP was investigated in the hamster after single and repeated oral administration. Single administration of ^{14}C-DCPP was performed in male and female hamsters. The dosing was performed at two dose levels, i.e. 2 mg/kg (low dose) and 200 mg/kg body weight (high dose). Excreta were collected in daily intervals and tissues were collected 96 h after administration. Blood and plasma kinetics was investigated after oral administration to male hamsters at the low dose level. Repeated administration of ^{14}C-DCPP was performed in male hamsters. Animals received 14 applications of radiolabelled test material on consecutive days. The ^{14}C-DCPP related residues in tissues and organs were determined by means of quantitative whole-body autoradiography technique. The excreted radioactivity was determined in urine and faeces at daily intervals.	
5.2	**Results and discussion**	Orally administered DCPP was rapidly and almost completely absorbed from the gastrointestinal tract into the systemic circulation. The daily administered dose was excreted mainly with the urine, accounting for about 80% of the daily dose and to a lower extent with the faeces, accounting in mean for 16% of the daily dose. The steady state in terms of excretion was reached just 1 day after the first administration. After single oral administration of ^{14}C-DCPP to male hamsters at the low dose level the maximum concentration level in blood and plasma was achieved 4 hour after administration. Thereafter the concentration decreased with an initial half-life (4-24 h) of about 6 hours and a terminal half life (24-96 h) of 26 and 36 hours for blood and plasma, respectively. The concentration levels in tissues and organs after administration at the low dose level were generally low, but still significantly above the LOQ level, except in brain of females. The highest concentrations were found in the bile fluid, plasma, kidneys, liver, and lungs. At the low dose level females revealed significantly lower residue levels as compared to males. At the high dose level the concentration in tissues and organs were correspondingly higher. The investigation of the metabolite pattern in urine and faeces revealed that DCPP was extensively metabolized. About 5-10% of the radioactivity was totally found as unchanged parent in urine and faeces. The majority of radiolabelled metabolites was excreted with the urine. The urinary metabolite pattern consisted of at least 16 metabolite fractions. It was dominated by fraction U11 (DCPP -glucuronide conjugate) accounting for 18-22% of the low dose and 31-61% of the	x

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

		high dose, followed by fraction U7 (glucuronide and sulphate conjugates of hydroxylated DCPP) accounting for about 10-12% of the low dose and 3-6% of the high dose. The metabolite pattern was very similar for both dose levels and both sexes with some quantitative differences. The major metabolite pathway was the forming of glucuronic acid conjugates of DCPP and/or hydroxylated DCPP. To a minor extent the sulphuric acid conjugates of DCPP and/or hydroxylated DCPP were found in the urine metabolite pattern. The metabolite pattern of urine and faeces, investigated at different time intervals during the dosing period were not essentially influenced by the multiple dosing regimen. The rates and routes of excretion, and the tissue distribution did not change upon multiple dosing as compared to single dosing. There is no indication for an accumulation of DCPP and/or related metabolites in tissues and organs.	
5.3	**Conclusion**	After single oral administration DCPP was rapidly and almost completely absorbed from the gastrointestinal tract into system circulation. The absorbed DCPP was extensively metabolized and mainly excreted with the urine. Absorption, metabolism, distribution and excretion profile was not essentially influenced by the gender or the dose level. The rates and routes of excretion, and the tissue distribution did not change upon multiple dosing as compared to single dosing. There is no indication for an accumulation of DCPP and/or related metabolites in tissues and organs.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	March 2010
Materials and Methods	3.3.2: Hasd:Han:AURA (repeated dose) 3.3.5: 106 g (males, repeated dose), typing error 3.4.1.2: 14 days (repeated dose) 3.4.1.3: single dose: 48 hours for blood-plasma kinetics, 96 hours for mass balance 3.5.2: single dose: blood: 0.25h, 0.5h, 1h, 2h, 4h, 8h, 24h, 32h, 48h;
Results and discussion	4.1: single dose: values refer to excretion and tissue residues, excluding exhaled air; repeated dose: values refer to excretion only. 4.2 Repeated dose: *Addition:* The plateau of blood and plasma concentration was reached just after the first administration (day 2) at a level of about 0.3 and 0.6 ppm DCPP equivalents. The half-life in blood of DCPP equivalents after the last dose was 24h and the terminal half-life was 32h. *4.3: Modification.* The actual extent of absorption may be even higher since the total recovery of the low dose level amounted only for 86% (males) and 84% (females), which is caused by a loss radioactivity in the wire grid of the metabolism cages. Also no measurements of exhaled air were performed. *4.6 Correction:* The amount of urinary daily excretion remained nearly constant until the end of dosing, accounting for 78% and 16% of the total ~~daily~~ dose for urine and faeces, respectively. Increase from 11 to 25% of the applied daily dose in feces was observed from day 1-2 to 14-15 after repeated dose. *5.2 Addition and deletion:* The highest concentrations were found in the bile fluid, plasma, kidneys, liver, lungs. At the low dose level females revealed lower residue levels as compared to males (Reason*: difference was not statistically evaluated*). At the high dose level the concentration in tissues and organs were correspondingly higher, highest concentrations were found in addition to the organs identified in the low dose group also in the adrenals.
Conclusion	5.3 *Replace*: ~~There is no indication for an accumulation of DCPP and/or related metabolites in tissues and organs.~~ Though terminal half-lives exceed 24 hours no tissue accumulation of DCPP equivalents were observed in the disposition study.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-1 **Mass balance after administration of a single dose**

Excretion [% of dose]					
Route of Administration		p.o.			
Group		1		2	
Sex		male	female	male	female
Dose	[mg/kg]	2.0	2.1	206	206
Urine					
	0 - 24 h	55.30	42.38	72.56	34.54
	24 - 48 h	5.00	7.29	4.37	7.92
	48 - 72 h	2.81	3.15	1.85	6.04
	48 - 96 h	1.96	3.05	1.39	4.07
	Subtotal	**65.07**	**55.86**	**80.17**	**52.56**
Feces					
	0 - 24 h	3.07	3.04	4.27	7.13
	24 - 48 h	0.99	0.74	0.43	1.58
	48 - 72 h	0.81	0.38	0.27	2.58
	72 - 96 h	0.67	0.46	0.37	1.33
	Subtotal	**5.54**	**4.62**	**5.34**	**12.62**
Cage Wash		**14.68**	**22.09**	**8.07**	**24.96**
Excretion		**85.28**	**82.57**	**93.58**	**90.15**
Tissue Residues		**1.21**	**1.44**	**0.64**	**1.67**
Recovery		**86.49**	**84.01**	**94.22**	**91.82**

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Figure A6_2-1: Blood/plasma toxicokinetics of ^{14}C-DCPP after single low-dose oral administration to male hamsters

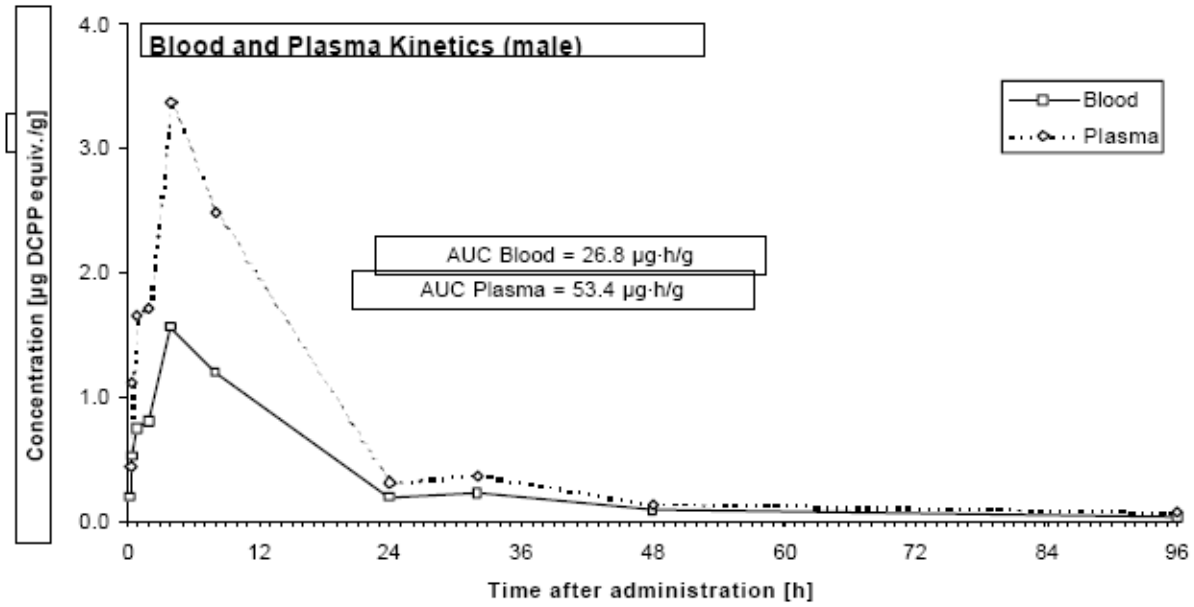
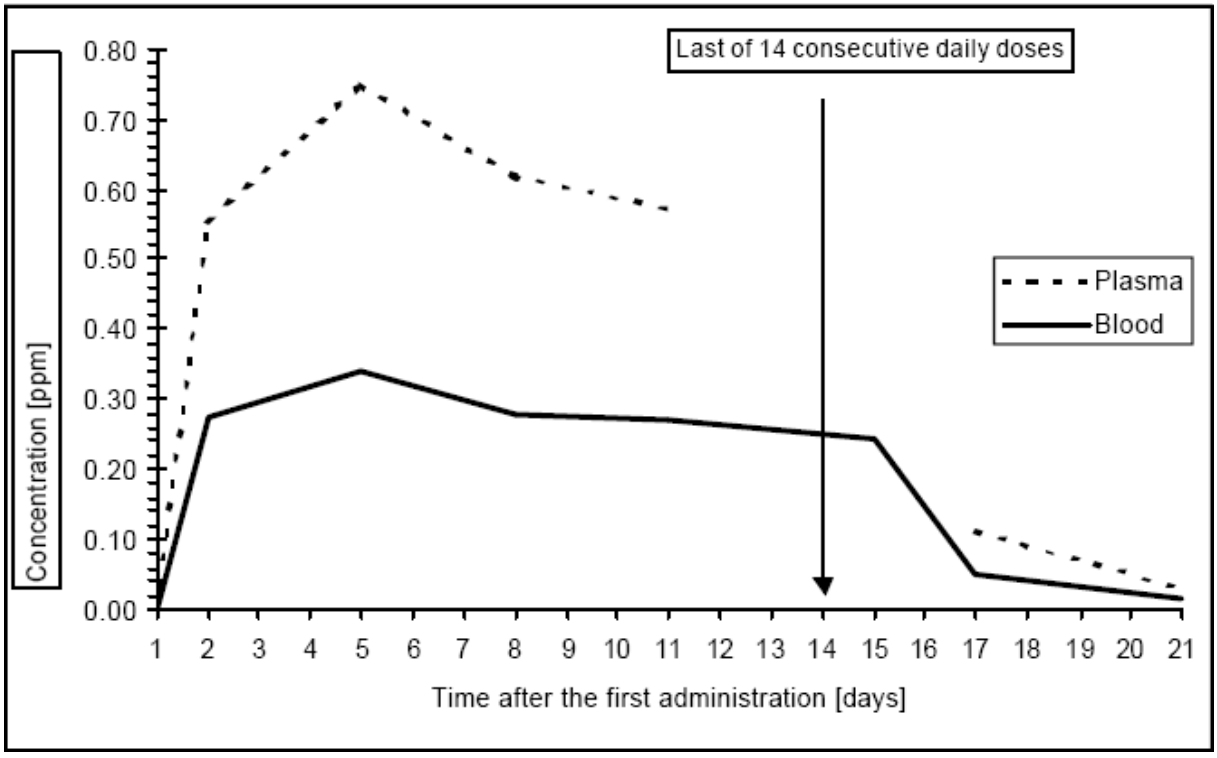


Figure A6_2-2: Blood/plasma toxicokinetics of ^{14}C-DCPP after repeated low-dose oral administration to male hamsters



Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-2 Tissue residues after a single oral administration

Tissue residues 96 hours after a single oral administration [ppm DCPP equivalents]						
Group	1			2		
Sex	male	female	LQ	male	female	LQ
Dose	2.0	2.1		206	206	
Adrenals	0.0099	0.0035	0.0027	0.801	2.176	0.105
Bile fluid	0.1142	0.0173	0.0094	3.424	2.491	0.393
Blood	0.0312	0.0060	0.0005	0.841	0.633	0.012
Brain	0.0013	<LOQ	0.0005	0.042	0.063	0.011
Epididymis	0.0085	-	0.0005	0.244	-	0.016
Fat (white)	0.0055	0.0019	0.0009	0.205	0.326	0.018
Femur	0.0060	0.0039	0.0002	0.198	0.557	0.006
Heart	0.0020	0.0087	0.0006	0.214	0.204	0.013
Kidneys	0.0496	0.0197	0.0005	0.854	1.132	0.012
Liver	0.0211	0.0104	0.0005	0.756	0.834	0.012
Lungs	0.0205	0.0041	0.0006	0.599	0.394	0.014
Muscle	0.0045	0.0041	0.0005	0.152	0.497	0.012
Ovaries	-	0.0039	0.0016	-	0.765	0.042
Pancreas	0.0092	0.0014	0.0007	0.203	0.187	0.016
Plasma	0.0695	0.0122	0.0005	1.866	1.258	0.012
Skin	0.0118	0.0061	0.0005	0.374	0.556	0.013
Spleen	0.0071	0.0018	0.0016	0.220	0.232	0.042
Testis	0.0047	-	0.0004	0.121	-	0.011
Thymus	0.0128	0.0045	0.0004	0.311	0.097	0.010
Uterus	-	0.0031	0.0004	-	0.690	0.010
Carcass	0.0291	0.0370	0.0003	4.621	4.621	0.008

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-3 Tissue residues after repeated oral administration

Concentration [ppm DCPP equivalents]								Calculated half life
Animal No.	1	2	3	4	5	6	7	$t_{½}$
Day of sacrifice	2	5	8	11	15	17	21	[h]
Adrenal	0.099	0.135	0.113	0.108	0.077	0.025	*0.004	35
Bile bladder	0.771	0.253	n.s.	n.s.	0.506	0.027	0.255	n.a.
Blood	0.305	0.303	0.306	0.272	0.222	0.049	*0.009	32
Bone	0.025	<LOQ	0.016	<LOQ	0.022	<LOD	<LOD	n.a.
Bone marrow	0.092	0.079	0.066	0.062	0.071	0.020	*0.001	24
Brain	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOD	<LOD	n.a.
Epididymis	0.052	0.051	0.032	0.057	0.044	0.042	*0.004	37
Fat white	0.030	0.030	0.018	0.025	0.035	<LOQ	=LOD	n.a.
Kidney cortex	0.224	0.329	0.202	0.237	0.214	0.040	0.022	48
Kidney medulla	0.143	0.193	0.118	0.143	0.133	0.032	*0.005	31
Liver	0.187	0.256	0.208	0.194	0.206	0.044	0.027	54
Lung	0.221	0.233	0.173	0.214	0.237	0.038	*0.008	31
Muscle	0.027	0.024	0.014	0.030	0.031	<LOQ	<LOD	n.a.
Myocardium	0.094	0.084	0.085	0.080	0.069	0.015	*0.002	31
Pancreas	0.048	0.043	0.049	0.087	0.090	<LOQ	<LOD	n.a.
Skin	0.048	0.092	0.074	0.058	0.063	0.018	*0.003	31
Spleen	0.050	0.079	0.065	0.072	0.046	<LOQ	<LOD	n.a.
Testis	0.042	0.044	0.030	0.033	0.022	0.025	*0.002	41
Thymus	0.026	0.040	0.030	0.040	0.021	<LOQ	<LOD	n.a.

LOQ = Limit of quantification n.a. = not applicable $t_{½}$ = Day 15-21
LOD = Limit of detection n.s.= no sample

* values were below LOQ, but were used as last value for half life calculation

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-4 Urinary metabolites after a single oral administration (LD: low dose, HD: high dose)

LD: Metabolite Pattern Urine (% of dose)							
	Males			Females			Assignment
Pool	G1/U1m	G1/U2m	Sum	G1/U1f	G1/U2f	Sum	
Sampling Time	0-24 h	24-48 h	0-48 h	0-24 h	24-48 h	0-48 h	
Metabolite Fraction							
U1	1.2	0.2	1.4	0.4	0.1	0.6	
U2	2.6	0.3	2.8	0.8	0.2	1.1	
U3	0.5	0.1	0.6	0.2	0.0	0.2	
U4	1.0	0.1	1.1	0.5	0.2	0.7	
U5	0.7	0.2	0.9	0.2	0.0	0.2	
U6	0.2	0.0	0.2	0.1	0.0	0.2	
U7	11.0	1.4	**12.4**	8.3	1.7	**10.0**	**M2, M5, M6**
U8	0.9	0.1	1.0	0.7	0.2	0.9	
U9	2.8	0.9	3.8	1.8	0.9	2.7	
U10	0.3	0.0	0.3	0.1	0.1	0.2	
U11	16.8	0.8	**17.6**	19.5	2.2	**21.7**	**M1**
U12	0.7	0.1	0.8	0.3	0.1	0.3	
U13	8.2	0.6	**8.8**	5.8	1.1	**6.9**	**M3**
U14	1.3	0.1	1.4	0.3	0.1	0.4	
U15	0.5	0.0	**0.5**	0.2	0.0	**0.2**	**M4**
U16	6.6	0.1	**6.7**	3.0	0.3	**3.3**	**DCPP**
Total*	**55.3**	**5.0**	**60.3**	**42.4**	**7.3**	**49.7**	

HD: Metabolite Pattern Urine (% of dose)							
	Males			Females			Assignment
Pool	G2/U1m	G2/U2m	Sum	G2/U1f	G2/U2f	Sum	
Sampling Time	0-24 h	24-48 h	0-48 h	0-24 h	24-48 h	0-48 h	
Metabolite Fraction							
U1	0.5	0.1	0.6	0.2	0.1	0.3	
U2	1.1	0.1	1.2	0.3	0.2	0.4	
U3	0.2	0.0	0.3	0.1	0.0	0.1	
U4	0.8	0.1	0.9	0.4	0.1	0.5	
U5	0.1	0.0	0.1	0.1	0.2	0.3	
U6	0.1	0.0	0.1	0.0	0.0	0.1	
U7	4.8	0.7	**5.5**	2.4	1.0	**3.4**	**M2, M5, M6**
U8	0.4	0.0	0.4	0.3	0.1	0.4	
U9	1.0	0.3	1.3	0.7	0.4	1.2	
U10	0.1	0.0	0.1	0.0	0.0	0.1	
U11	58.3	2.6	**60.9**	26.3	4.4	**30.7**	**M1**
U12	0.1	0.0	0.2	0.1	0.1	0.2	
U13	2.0	0.2	**2.2**	1.4	0.5	**1.9**	**M3**
U14	0.1	0.0	0.1	0.1	0.1	0.1	
U15	1.1	0.0	**1.1**	0.3	0.1	**0.4**	**M4**
U16	1.9	0.1	**2.0**	1.8	0.7	**2.5**	**DCPP**
Total*	**72.6**	**4.4**	**77.0**	**34.5**	**7.9**	**42.5**	

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

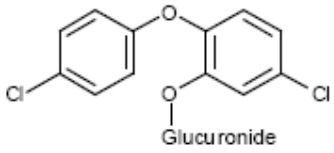
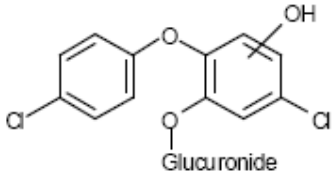
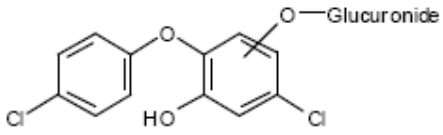
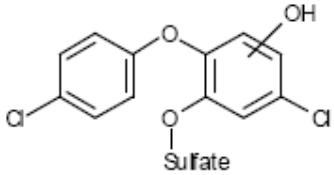
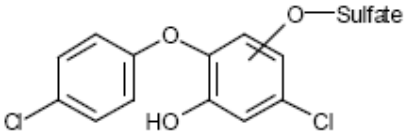
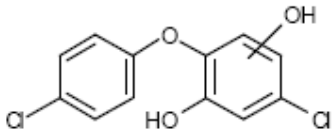
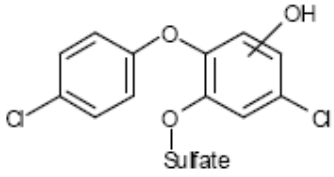
Table A6_2-5 Urinary metabolites after repeated oral administration

Metabolite Pattern Urine (% of daily dose)					
Pool	G1-U1m	G1-U2m	G1-U4m	G1-U3m	Assignment
Sampling Time	Day 1-2	Day 7-8	Day 13-14	Day 14-15	
Metabolite Fraction					
U1	2.75	3.09	1.33	1.72	
U2	2.75	2.90	2.09	2.29	
U3	0.00	0.00	0.36	0.00	
U4	0.15	0.00	1.17	0.00	
U5	1.72	2.41	0.43	1.07	
U6	0.77	1.43	1.25	0.87	
U7	**12.23**	**16.70**	**13.94**	14.02	DCPP-hydroxy-glucuronide
U8	1.23	1.37	0.00	2.12	
U9	4.40	4.64	4.03	3.50	
U10	0.00	0.00	0.00	0.00	
U11	**40.28**	**38.10**	**42.09**	12.34	DCPP-glucuronide
U12	0.00	0.00	0.17	0.00	
U13	6.31	4.56	3.51	6.52	
U14	0.49	0.19	0.00	0.31	
U15	0.36	0.55	0.29	0.00	
U16	**0.88**	**1.70**	**2.53**	20.75	unchanged DCPP
Total	**74.31**	**77.64**	**73.20**	**65.51**	

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-6 Urinary metabolites

Metabolite Fraction	Metabolite Number	Name	Structure
U11	M1	DCPP-glucuronide	
	M2 (major)	Hydroxy-DCPP-glucuronide	
U7	M5 (major)	DCPP-hydroxy-glucuronide	
	M6 (minor)	Hydroxy-DCPP-sulfate or DCPP-hydroxy-sulfate	 or 
U13	M3	Hydroxy-DCPP	
U15	M4	DCPP-sulfate	

Section A6.2 Absorption, distribution, metabolism and excretion

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-7 Faecal metabolites after a single oral administration (LD: low dose, HD: high dose)

LD: Metabolite Pattern Feces (% of dose)							
	Males			Females			Assignment
Pool	G1/F1m	G1/F2m	Sum	G1/F1f	G1/F2f	Sum	
Sampling Time	0-24 h	24-48 h	0-48 h	0-24 h	24-48 h	0-48 h	
Metabolite Fraction							
F1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
F2	<0.1	<0.1	0.1	0.1	<0.1	0.1	
F3	<0.1	<0.1	0.1	0.1	<0.1	0.2	
F4	0.4	0.4	0.8	0.5	0.2	0.7	
F5	2.4	0.3	**2.7**	2.1	0.3	**2.4**	**DCPP**
F6	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
F7	<0.1	<0.1	0.1	<0.1	<0.1	0.1	
F8	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	
Extract 1	**2.9**	**0.9**	**3.8**	**2.9**	**0.6**	**3.5**	
Non-extractable	0.2	0.1	0.3	0.1	0.1	0.2	
Total	**3.1**	**1.0**	**4.1**	**3.0**	**0.7**	**3.8**	

HD: Metabolite Pattern Feces (% of dose)							
	Males			Females			Assignment
Pool	G2/F1m	G2/F2m	Sum	G2/F1f	G2/F2f	Sum	
Sampling Time	0-24 h	24-48 h	0-48 h	0-24 h	24-48 h	0-48 h	
Metabolite Fraction							
F1	0.0	0.0	0.0	0.0	0.0	0.1	
F2	0.0	0.0	0.1	0.1	0.1	0.2	
F3	0.0	0.0	0.1	0.0	0.0	0.1	
F4	0.3	0.1	0.5	0.4	0.1	0.5	
F5	3.7	0.2	**3.9**	6.4	1.1	**7.6**	**DCPP**
F6	0.0	0.0	0.0	0.0	0.0	0.0	
F7	0.0	0.0	0.0	0.0	0.0	0.1	
F8	0.0	0.0	0.0	0.0	0.0	0.1	
Extract 1	**4.2**	**0.4**	**4.6**	**7.1**	**1.5**	**8.6**	
Non-extractable	0.1	0.0	0.1	0.1	0.1	0.2	
Total	**4.3**	**0.4**	**4.7**	**7.1**	**1.6**	**8.7**	

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

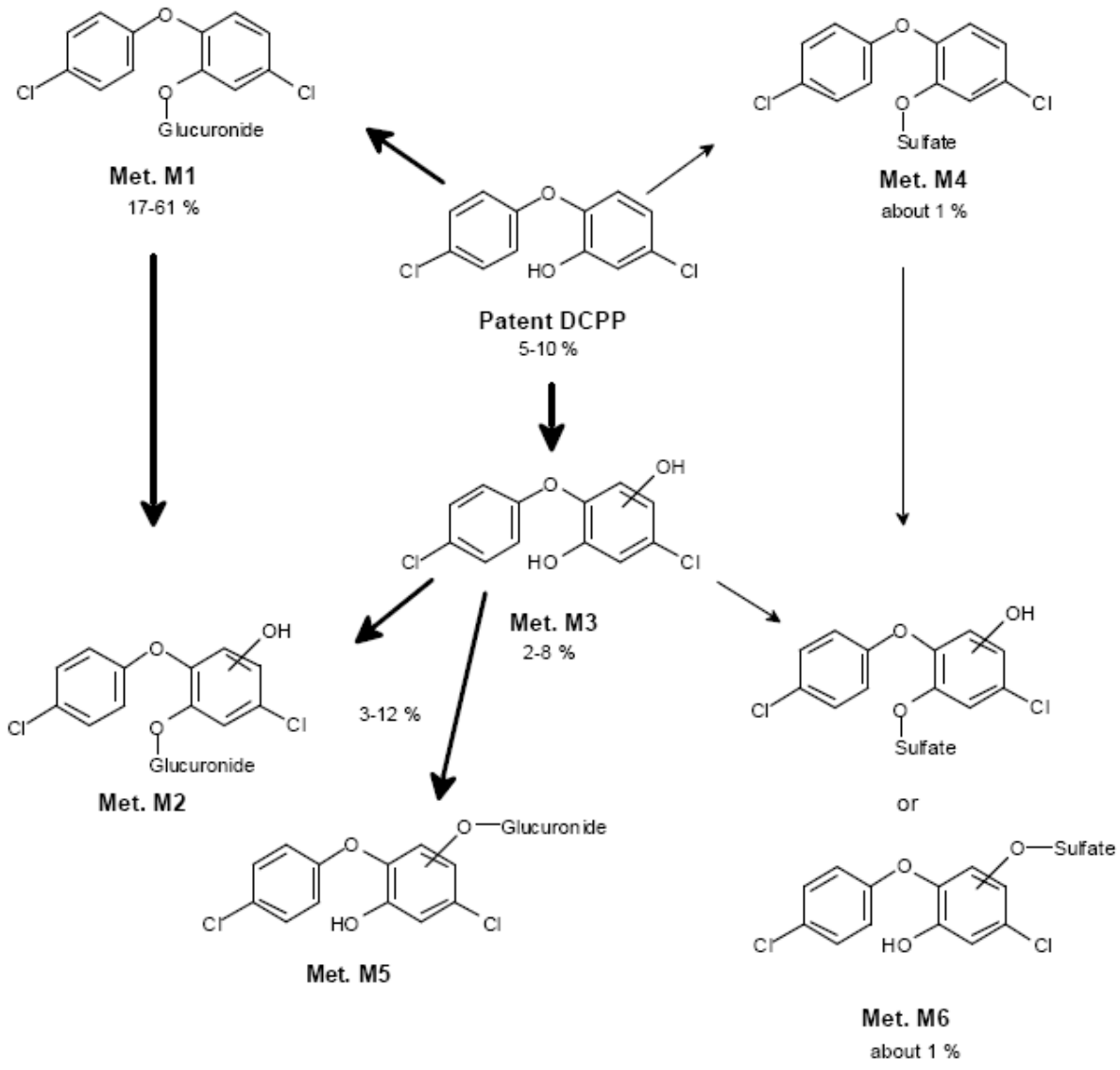
Table A6_2-8 Faecal metabolites after a repeated oral administration

Metabolite Pattern Feces (% of daily dose)				
Pool	G1-F1m	G1-F2m	G1-F3m	Assignment
Sampling Time	Day 1-2	Day 7-8	Day 14-15	
Metabolite Fraction				
F1	0.00	0.00	0.35	
F2	0.19	1.70	1.70	
unknown	0.12	1.90	1.61	
F3	0.19	0.56	0.42	
F4	1.62	3.22	3.88	
F5	**8.29**	**11.32**	**15.68**	**unchanged DCPP**
F6	0.10	0.00	0.00	
F7	0.21	0.00	0.00	
Extract 1	**10.73**	**18.70**	**23.64**	
Non-extractable	0.34	0.84	0.88	
Total	**11.07**	**19.54**	**24.52**	

Section A6.2 **Absorption, distribution, metabolism and excretion**

Annex Point IIA VI.6.2 6.2 ADME of DCPP in Syrian hamsters

Table A6_2-8 Proposed metabolic pathway of ^{14}C-DCPP in hamsters



Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

Official use only

1 REFERENCE

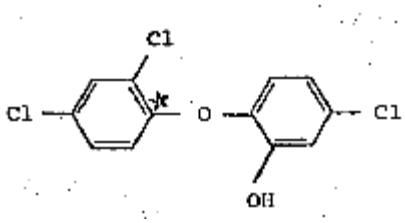
1.1	**Reference**	[redacted] (1972) Study of pharmacokinetics and metabolism in mouse, rat, rabbit and dog. Pharma Research, Pharmacological Chemistry, [redacted] Unpublished Report No. 33/1972, 1st Dec 1972, G, BPD ID A6.02_03
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since supplier is study owner
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; the study was conducted in the early seventies, i.e. prior to the implementation of currently accepted testing guidelines.
2.2	**GLP**	No; GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	Not applicable.

3 MATERIALS AND METHODS

3.1	**Test material**	Triclosan
3.1.1	^{14}C-labelled Triclosan	
3.1.1.1	Radiolabelling	^{14}C



3.1.1.2	Specific activity	13.25 µCi/mg
3.1.1.3	Purity	99.5% chemically and radiochemically pure (thin layer chromatography)
3.1.1.4	Stability	No data
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	Not specified
3.2.4	Sex	male

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

3.2.5	Age/weight at study initiation	10 and 14 kg, respectively
3.2.6	Number of animals per group	2 animals were sued
3.2.7	Control animals	No
3.2.8	Holding conditions	Not specified
3.3	**Administration/ Exposure**	
3.3.1		**Single intravenous injection**
3.3.1.1	Application	injection
3.3.1.2	Dose	5 mg/kg bw
3.3.1.3	Vehicle	Sodium salt in aqueous solution
3.3.2		**Orally, 14 days after i.v.**
3.3.2.1	Application	test item offered in a gelatine capsule
3.3.2.2	Doses	5 mg/kg bw
3.3.2.3	Vehicle	Sodium salt in aqueous solution

3.3.3 Blood sampling

Total radioactivity count expressed in µg/mL of blood (animal 1 and animal 2)		
Samples	Dose	Time points for sampling
Blood sampling after i.v. administration	5 mg/kg bw (^{14}C-labelled TS)	10, 30 min, 1, 2, 4, 8, 24, 48 h
Blood sampling after oral administration	5 mg/kg bw (^{14}C-labelled TS)	10, 30 min, 1, 2, 4, 8, 24, 48 h
Unchanged parent compound recovery in blood expressed µg/mL and as % (only animal 1)		
Blood sampling after i.v. administration	5 mg/kg bw (^{14}C-labelled TS)	1, 2, 8 h
Blood sampling after oral administration	5 mg/kg bw (^{14}C-labelled TS)	1, 2, 8 h

3.3.4 Urine and faeces sampling

Excretion of radioactivity in urine and faeces from each treated animal, after i.v. and oral treatment, respectively		
Samples	Dose	Time points or intervals (minutes and hours, post-treatment)
Urine (N = 2)	5 mg/kg bw (^{14}C-labelled TS)	0-6 h; 6-24 h; 24-48 h; 48-72 h; 72-96 h; 96-120 h
Feces (N = 2)	5 mg/kg bw (^{14}C-labelled TS)	0-24 h; 24-48 h; 48-72 h; 96-120 h

3.4 Analytical methods

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

3.4.1 Radioactivity measurement

Liquid scintillation counting (Packard Model 4000 and 3380 liquid scintillation counters) and thin layer scanning (Berthold Radioactivity Scanner or a Packard "Actigraph").

Preparation of samples for analysis:

Sample	Preparation prior to/for measurement
Urine	none; directly measured
Faeces	homogenization, freeze-drying, combustion of aliquots
Blood	0.5 mL samples dried on filter tablets (2 x 2 cm), combustion

3.4.2 Metabolites identification

3.4.2.1 Urine and faecal extracts

Urine and faecal extracts were adjusted to a pH of 1-2 with HCl and extracted several times with petroleum ether. The extracts were identified by thin layer chromatography and compared with authentic material.

3.4.2.2 Glucuronide content in urine

The glucuronide content of the urine was determined by incubation of the urine for 24 hours at 37 °C, pH 6.8, with beta-glucuronidase. After acidification, the aglycone was extracted and further processed as described above (3.4.2.1).

3.4.2.3 Extraction of faeces

Aliquots of the freeze-dried faeces were vigorously shaken in methanol, methanol/water, water and acetone successively; the concentrated extracts were then further processed (see 3.4.2.1)

4 RESULTS AND DISCUSSION

4.1 RECOVERY IN BLOOD

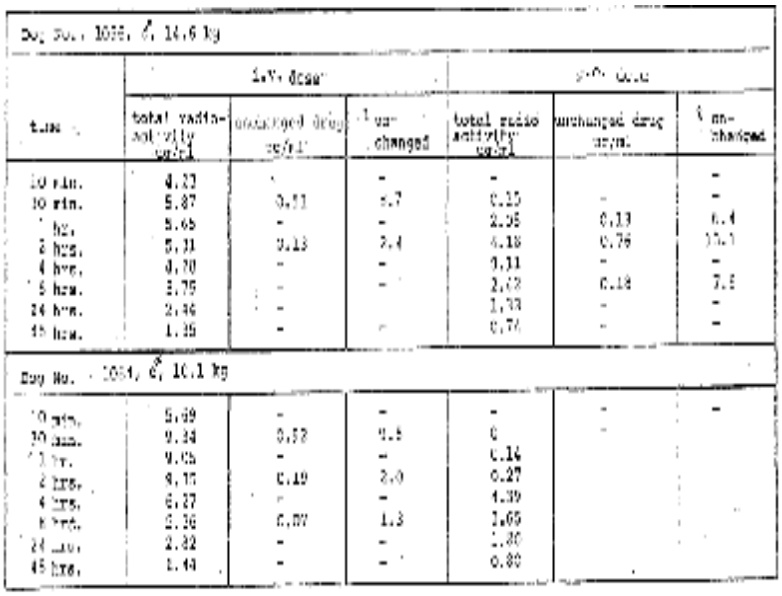
Similar as for the rat, the blood concentrations of the radiolabeled material in dog increased after the i.v. injection. In this case, the increase was from 4.2 and 5.7 µg/mL after 10 minutes to 5.7 and 9.1 µg/mL after one hour, for the first and the second animal, respectively. The percentage of the total radioactivity related to unchanged parent compound was reported to amount to ca. 10% (average 0.7 µg/mL) after 30 minutes following treatment, decreasing to about 1% (0.07 µg/mL) after 8 hours. No biological half-life could be determined, due to an insufficient number of determinations.

Following oral administration, the onset of absorption was reported to be relatively slow. Maximum concentrations of 4.2 to 4.4 µg/mL were reached between 2 and 4 hours after administration. The percentage of unchanged parent compound was quite similar as after i.v. injection.

Blood concentrations in dog after i.v. injection and after oral administration of radiolabeled compound (5 mg/kg bw), as total radioactivity count and unchanged parent compound in µg/mL (table 10 from study report):

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2



time	i.v. dose: total radioactivity µg/ml	i.v. dose: unchanged drug µg/ml	i.v. dose: % unchanged	p.o. dose: total radioactivity µg/ml	p.o. dose: unchanged drug µg/ml	p.o. dose: % unchanged
10 min.	6.22	-	-	-	-	-
30 min.	5.87	0.51	8.7	0.15	-	-
1 hr.	5.65	-	-	2.05	0.13	6.4
2 hrs.	5.31	0.13	2.4	4.18	0.79	18.1
4 hrs.	4.70	-	-	3.11	-	-
8 hrs.	3.75	-	-	2.42	0.18	7.5
24 hrs.	2.46	-	-	1.33	-	-
48 hrs.	1.35	-	-	0.74	-	-
Dog No. 1004, ♂, 10.1 kg						
10 min.	5.49	-	-	-	-	-
30 min.	5.34	0.52	9.8	0		
1 hr.	4.85	-	-	0.14		
2 hrs.	4.17	0.19	3.0	0.27		
4 hrs.	3.27	-	-	1.39		
8 hrs.	2.36	0.07	1.3	1.69		
24 hrs.	2.32	-	-	1.20		
48 hrs.	1.44	-	-	0.80		

4.2 ABSORPTION

For determination of the absorption, the total surface areas under the blood concentration curves obtained after oral and intravenous treatment with the radiolabelled test item were compared. The comparison revealed that the test item was absorbed to about 50 to 65%.

4.3 EXCRETION

4.3.1 Recovery in urine

After the intravenous injection, the first dog excreted about 13% of the radioactivity via urine over 120 hours; however it was reported that during sampling between 24 and 48 h, urine was lost, with no more details provided in the study report. The second dog excreted about 18%. After oral administration, the first and the second dog excreted about 8 and 9% of the radioactivity via urine over 120 hours, respectively.

Radioactivity recovery in the urine of dog after i.v. (5 mg/kg bw) and after oral administration (5 mg/kg bw) of radiolabeled test item (% of dose)

Time * (h)	Animal 1		Animal 2	
	i.v.	oral	i.v.	oral
0-6	3	1.3	0.9	0.7
6-24	5.3	3.1	7.9	4.3
24-48	2.3 (urine lost)	2.1	5.0	2.3
48-72	1.2	1.2	2.2	1.0
72-96	0.6	0.6	1.3	0.5
96-120	0.5	-	0.4	-
0-120	12.9	8.3	17.7	8.8

*; Time interval after treatment (hours)

4.3.2 Recovery in faeces

After the intravenous injection, the first dog excreted about 67% of the radioactivity in the faeces collected between 0 and 120 hours; the second dog excreted about 70%. After oral administration, the first and the second dog excreted about 70 and 71% of the radioactivity in the faeces collected between 0 and 120 hours, respectively.

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

Radioactivity recovery in the faeces of dog after i.v. (5 mg/kg bw) and after oral administration (5 mg/kg bw) of radiolabeled test item (% of dose)				
Time	Animal 1		Animal 2	
	i.v.	oral	i.v.	oral
0-24 h	40.2	49.8	33.7	45.2
24-48 h	8.3	10.8	12.4	16.5
48-72 h	9.9	6.6	15.1	4.5
72-96 h	9.0*	0.5	8.9*	2.1*
96-120 h		2.1		
0-120 h	67.4	69.8	70.1	71.4
*; there was no clear assignment of these 3 values to a time interval in the study report; no explanation was provided.				

4.3.3 Conclusion

Similar as for the rat, the main way of excretion in dog was via faeces (> 65% or recovery) whereas excretion via the urine was comparatively low (< 20%). The recovery values obtained over 120 hours for both, faeces and urine were in the range of 78 to 88%, thus indicating that excretion was not fully achieved 120 hours, i.e. five days. By comparing recovery after i.v. application and after oral administration, it could be evidenced that about 50 to 65% of the initially administered dose referring to radioactivity was absorbed from the gut.

4.4 METABOLITES

4.4.1 In urine

Following i.v. injection about twice as much radioactive material was excreted in the urine of the dog as after oral administration (16% as opposed to 8.5%). Approximately half of the radioactivity in the urine after the i.v.injection was reported to originate from unchanged parent compound. About 15 to 20% of the radioactivity referred to glucuronides, with only a small part of the aglycones consisting of parent compound whereas the greater part referred to highly polar compounds, as shown by thin layer chromatography (TLC).

Following oral administration, 30% of the radioactivity detected in the urine was related to the unchanged parent compound. About 30 to 40% were identified as referring to the glucuronide of the parent compound and a small additional percentage was related to the glucuronides of more highly polar metabolites (TMC).

4.4.2 In faeces

About 85% of the radioactive material present in the faeces after i.v. injection of the radiolabeled test material could be extracted; therefrom, 72% of this amount corresponding to 60% of the radioactivity in faeces was identified as unchanged parent compound.

Following oral administration, 100% of the radioactive material present in the faeces was extracted; therefrom, 87% corresponded to unchanged parent compound. The greater quantity of unchanged substance recovered in the faeces after oral administration is in agreement with the calculated absorption rate of 50-65%. Only a small percentage of glucuronides were found in the faeces

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

4.5	**CONCLUSION**	The main findings of the present study can be summarized as follows: The comparison of the total surface areas under the blood concentration curves obtained after oral and intravenous administration indicates an absorption level of about 50 to 65%. Similarly as for the rat, blood concentration of radioactivity first showed a delayed increase, reaching 10% of total radioactivity after 30 minutes, followed by a decrease to about 1% after 8 hours. Also similar as for the rat, the main way of excretion in dog was via faeces (> 65% or recovery) whereas excretion via the urine was comparatively low (< 20%). The recovery values obtained over 120 hours for both, faeces and urine were in the range of 78 to 88%, thus indicating that excretion was not fully achieved 120 hours, i.e. five days. By comparing recovery after i.v. application and after oral administration, it could be evidenced that about 50 to 65% of the initially administered dose referring to radioactivity was absorbed from the gut. With respect to metabolite identification, the metabolism of the substance in the dog was found to differ depending on the route of administration. In fact, urine contained more unchanged parent compound after i.v. injection than after oral administration. After i.v. injection, about 60% of the radioactivity detected in the faeces was related to unchanged parent compound whereas after oral administration, 85 to 90% of the radioactivity was related to parent compound. Only small amounts of glucuronides were detected.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	An ADME study with radiolabelled triclosan was conducted in the early seventies, with rats, mice, rabbits and dogs. The study was conducted prior to the implementation of the current testing guidelines and GLP. The present summary refers only to the experiments conducted with dogs. Two animals were used. Each animal received a single i.v. injection of radiolabeled test item and, after 14 days a single gelatine capsule containing the radiolabeled test item, offered orally. For each application, the dose level was 5 mg/kg bw. The absorption, excretion and metabolism of the test item in dog were investigated. Radioactivity measurement and metabolite identification were principally based on liquid scintillation counting and thin layer chromatography.

Section A6.2_03 Absorption, excretion and metabolism in dog

Annex Point IIA6.2

5.2	**Results and discussion**	Based on results of the present study, following main findings can be retained: In dog, triclosan is well absorbed following oral uptake, since an absorption level up to 65% could be shown. Based on the decline of test item concentration in the blood, as for the rat, an enterohepatic circulation of the test item is expected. Elimination was less rapid when compared to rat, since the recovery values after 120 hours for both, faeces and urine were in the range of 78 to 88%, indicating that excretion was not fully achieved after 5 days. As for rat, the main way of excretion is via the faeces (> 65% or recovery) whereas excretion via the urine is comparatively low (< 20%). With respect to metabolite identification, the metabolism of triclosan in dog differs depending on the route of administration. In fact, urine contained more unchanged parent compound after i.v. injection than after oral administration. After i.v. injection, about 60% of the radioactivity detected in the faeces was related to unchanged parent compound whereas after oral administration, 85 to 90% of the radioactivity was related to parent compound. Only small amounts of glucuronides were detected.
5.3	**Conclusion**	
5.3.1	Reliability	The study was conducted in the early seventies, i.e. prior to the implementation of current testing guidelines and GLP. The study report shows some deficiencies when comparing to current quality criteria. Nevertheless, the methods which were applied are considered suitable, and the results of the different experiments taken together are scientifically acceptable, allowing a comprehensive insight with respect to the fate of triclosan in dog. Therefore, a RL 2 is given.
5.3.2	Deficiencies	- Regarding radioactivity recovery in the faeces of dog after i.v. and after oral treatment, for some values there was no clear assignment to a time interval in the study report, with no explanation was provided (see table in 4.3.2). - The description of substance preparation was poor. - The description of the study design in the method part of the study report was poor.

Evaluation by Competent Authorities

Date	2013-11
Materials and Methods	Agree with applicant´s summary
Results and discussion	Agree with applicant´s conclusion
Conclusion	Agree with applicant´s conclusion
Reliability	2
Acceptability	acceptable
Remarks	

Section A6.2_03 Distribution in mice followin single iv injection

Annex Point IIA6.2

Official use only

1 REFERENCE

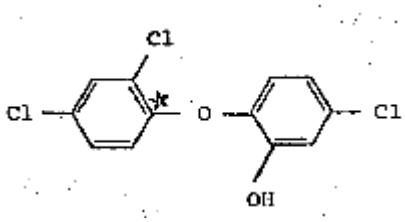
1.1	**Reference**	[redacted](1972) Study of pharmacokinetics and metabolism in mouse, rat, rabbit and dog. Pharma Research, Pharmacological Chemistry, [redacted] Unpublished Report No. 33/1972, 1st Dec 1972, G, BPD ID A6.02_03
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since supplier is study owner
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; the study was conducted in the early seventies, i.e. prior to the implementation of currently accepted testing guidelines.
2.2	**GLP**	No; GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	Not applicable.

3 MATERIALS AND METHODS

3.1	**Test material**	Triclosan
3.1.1.1	Radiolabelling	^{14}C



3.1.1.2	Specific activity	13.25 µCi/mg
3.1.1.3	Purity	99.5% chemically and radiochemically pure (thin layer chromatography)
3.1.1.4	Stability	No data
3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	MF 2 (BALB/C x MJW) SPF strain
3.2.3	Source	[redacted]
3.2.4	Sex	male
3.2.5	Age/weight at study initiation	20 ± 1 g

Section A6.2_03 Distribution in mice followin single iv injection

Annex Point IIA6.2

3.2.6	Number of animals per group	Distribution assay: 8 mice For whole body autoradiography: 4 mice
3.2.7	Control animals	No
3.2.8	Holding conditions	Not specified
3.3	**Administration/ Exposure**	**Single dose injection into the caudal vein**
3.3.1	Application	Injection in the caudal vein
3.3.2	Doses	10 mg/kg bw (0.2 mL/0.1 mL vehicle)
3.3.3	Vehicle	Sodium carbonate 1 N
3.3.4	Distribution	The total radioactivity levels in the organs were determined in 2 mice at each of four different times after treatment.
3.3.4.1	Sampling time points	For organ sampling, 2 animals of the treated group were sacrificed after 1 minute, 5 minutes, 30 minutes and 2 hours following injection, respectively.
3.3.4.2	Organs and tissues	Blood, plasma, heart, muscle, brain, spinal cord, fat (brown and white), testis, lung, adrenals, spleen, thymus, thyroid, salivary gland, pancreas, liver, gall bladder with contents, and kidney.
3.3.5	Autoradiography	Whole-body sections were prepared from treated mice sacrificed after 1 minute, 30 minutes, 2 hours and 24 hours following injection, respectively.
3.4	**Analytical methods**	
3.4.1	Radioactivity measurement	Liquid scintillation counting (Packard Model 4000 and 3380 liquid scintillation counters). Preparation of samples for analysis:

Sample	Preparation prior to/for measurement
Organs	homogenization, aliquots dissolved in hyamine hydroxide (45°C over 15 hrs) or combustion of aliquot parts up to 100 mg (according to Kalberer and Rutschmann, Helv Chim Acta 44:1956, 1961)
Blood	0.5 mL samples dried on filter tablets (2 x 2 cm), combustion

3.4.2	Whole-body autoradiography	Following sacrifice (see 3.3.5), the mice were deep-frozen at -80 °C. Whole-body sections were obtained by cutting on a microtome at -20 °C. The sections were then kept in contact with the photographic plates for 15 days at -20°C.

4 RESULTS AND DISCUSSION

4.1 DISTRIBUTION

Section A6.2_03 Distribution in mice followin single iv injection

Annex Point IIA6.2

4.1.1 Recovery of radioactivity (^{14}C)

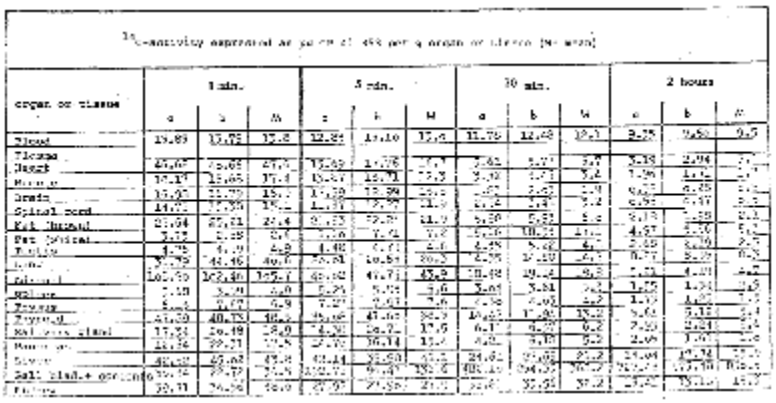
After 1 minute following injection: comparatively high concentrations of radioactivity were recovered in the adrenals (about 106 µg/g of tissue), the thyroid (about 48 µg/g of tissue), the heart (about 48 µg/g of tissue), the liver (about 44 µg/g of tissue) and the lungs (about 41 µg/g of tissue), followed by the kidneys (about 38 µg/g of tissue), the gall bladder with content (about 36.5 µg/g of tissue) and the brown fat (about 24 µg/g of tissue).

After 5 minutes following injection: the concentrations of radioactivity in all organs reported above were found to decrease rapidly except for the gall bladder with contents, where a rapid increase was seen (about 137 µg/g of tissue).

After 30 minutes following injection: the concentrations in the organs reported above further decreased rapidly. The highest concentration of radioactivity was still found in the gall bladder including contents, having reached about 368 µg/g of tissue, followed by the kidneys (about 32 µg/g of tissue) and the liver (about 26 µg/g of tissue).

After 2 hours following injection: the concentration of radioactivity in the gall bladder further increased up to about 850 µg/g of tissue. In the liver and the kidneys about 16 and 13 µg/g of tissue were recovered, respectively.

Details of ^{14}C- recovery in organs and tissues of male mice at different time points following single i.v. injection of radiolabelled test item (Table 1 of study report):



4.1.2 Analysis of the whole body section

After 2hours following treatment: radioactivity recovery in the gall bladder was found to be very high.

After 24 hours following treatment: radioactivity was predominantly recovered in the gut.

4.1.3 Conclusion

The high recovery of radioactivity in the gall bladder indicates that the test item and/or metabolites are mostly eliminated via the bile. This is supported by the slow rate of decline of the radioactivity concentration in blood, which further supports the assumption that that a marked entero-hepatic circulation takes place.

5 APPLICANT'S SUMMARY AND CONCLUSION

Section A6.2_03 Distribution in mice followin single iv injection

Annex Point IIA6.2

5.1	**Materials and methods**	An ADME study with radiolabelled triclosan was conducted in the early seventies, with rats, mice, rabbits and dogs. The study was conducted prior to the implementation of the current testing guidelines and GLP. The present summary refers only to the experiments conducted with mice. The mice received a single injection of ^{14}C-labelled test item in the caudal vein; the dose level was 10 mg/kg bw; sodium carbonate was used as vehicle. A distribution assay was conducted, with 2 mice being sacrificed after 1 minute, 5 minutes, 30 minutes and 2 hours following injection, respectively. Blood, plasma, heart, muscle, brain, spinal cord, fat (brown and white), testis, lung, adrenals, spleen, thymus, thyroid, salivary gland, pancreas, liver, gall bladder with contents, and kidney were sampled and prepared for radioactivity measurement (liquid scintillation counting). In a second assay, whole-body sections were prepared for autoradiography from treated mice sacrificed after 1 minute, 30 minutes, 2 hours and 24 hours following injection, respectively. Following sacrifice, the mice were deep-frozen at -80 °C, and whole-body sections were cut at -20 °C using a microtome and were then kept in contact with the photographic plates for 15 days at -20°C.
5.2	**Results and discussion**	The distribution assay revealed that after 1 minute following injection comparatively high concentrations of radioactivity were present in the adrenals (about 106 µg/g of tissue), the thyroid (about 48 µg/g of tissue), the heart (about 48 µg/g of tissue), the liver (about 44 µg/g of tissue) and the lungs (about 41 µg/g of tissue), followed by the kidneys (about 38 µg/g of tissue), the gall bladder with content (about 36.5 µg/g of tissue) and the brown fat (about 24 µg/g of tissue). Already after 5 minutes following injection, the concentrations of radioactivity in all organs reported above were found to decrease rapidly except for the gall bladder (incl. contents), where a rapid increase was seen (about 137 µg/g of tissue). After 30 minutes following injection: the concentrations in the organs reported above further decreased rapidly. The highest concentration of radioactivity was still found in the gall bladder including contents, having reached about 368 µg/g of tissue, followed by the kidneys (about 32 µg/g of tissue) and the liver (about 26 µg/g of tissue). After 2 hours following injection: the concentration of radioactivity in the gall bladder further increased up to about 850 µg/g of tissue. In the liver and the kidneys about 16 and 13 µg/g of tissue were recovered, respectively. The results of the whole-body autoradiography were in accordance with those given above, with very high radioactivity recovery seen in the gall bladder after 2hours following treatment. Moreover, autoradiography revealed that after 24 hours following treatment, radioactivity was predominantly recovered in the gut. Thus, the results obtained from the studies with mice revealed that the test item and/or metabolites are mostly eliminated via the bile, with a marked entero-hepatic circulation taking place.
5.3	**Conclusion**	
5.3.1	Reliability	The study was conducted in the early seventies, i.e. prior to the implementation of current testing guidelines and GLP. The study report shows some deficiencies when comparing to current quality criteria. Nevertheless, the methods which were applied are considered suitable, and the results of the different experiments taken together are

Section A6.2_03 Distribution in mice followin single iv injection

Annex Point IIA6.2

		scientifically acceptable, allowing a comprehensive insight with respect to the distribution of triclosan in mice following i.v. injection. Therefore, a RL 2 is given.
5.3.2	Deficiencies	The description of the study design in the method part of the study report was relatively poor.

Evaluation by Competent Authorities	
Date	2013-11
Materials and Methods	Agree with applicant´s summary
Results and discussion	Agree with applicant´s conclusion
Conclusion	Agree with applicant´s conclusion
Reliability	2
Acceptability	acceptable
Remarks	

Section A6.2_03

Excretion via urine and faeces in rabbit, following single oral administration by gavage

Annex Point IIA6.2

Official use only

1 REFERENCE

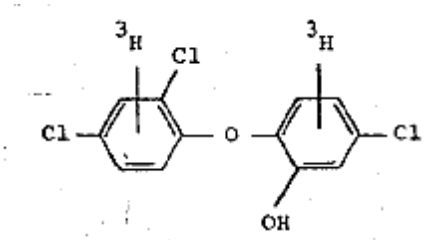
1.1	**Reference**	[redacted](1972) Study of pharmacokinetics and metabolism in mouse, rat, rabbit and dog. Pharma Research, Pharmacological Chemistry, [redacted] Unpublished Report No. 33/1972, 1st Dec 1972, G, BPD ID A6.02_03
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since supplier is study owner
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; the study was conducted in the early seventies, i.e. prior to the implementation of currently accepted testing guidelines.
2.2	**GLP**	No; GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	Not applicable.

3 MATERIALS AND METHODS

3.1	**Test material**	Triclosan
3.1.1	Tritiated Triclosan	
3.1.1.1	Radiolabelling	Tritiated



3.1.1.2	Specific activity	62.7 µCi/mg
3.1.1.3	Purity	99% chemically and radiochemically pure (isotope dilution analysis and thin layer chromatography)
3.1.1.4	Stability	Proven by testing in neutral, acid and alkaline media; ^{3}H-exchangeability was < 1%.
3.1.1.5	Stability	No data
3.2	**Test Animals**	
3.2.1	Species	Rabbit
3.2.2	Strain	mixed-bred rabbits, no further details given
3.2.3	Source	Not specified

Section A6.2_03 Excretion via urine and faeces in rabbit, following single oral administration by gavage

Annex Point IIA6.2

3.2.4	Sex	male
3.2.5	Age/weight at study initiation	2 – 3 kg
3.2.6	Number of animals per group	3 animals
3.2.7	Control animals	No
3.2.8	Holding conditions	In metabolism cages; water available ad libitum; food withdrawn 15 hours before test initiation and given back after 2 hours following test item administration.
3.3	**Administration/ Exposure**	
3.3.1		**Oral**
3.3.1.1	Application	Gavage
3.3.1.2	Doses	5 and 50 mg/kg bw
3.3.1.3	Vehicle	Not specified
3.3.1.4	Sampling	Urine and faeces were collected in 24-hour portions over 72 hours following treatment.
3.4	**Analytical methods**	
3.4.1	Radioactivity measurement	Liquid scintillation counting (Packard Model 4000 and 3380 liquid scintillation counters) and thin layer scanning (Berthold Radioactivity Scanner or a Packard "Actigraph"). Preparation of samples for analysis:

Sample	Preparation prior to/for measurement
Urine	none; directly measured
Faeces	homogenization, freeze-drying, combustion of aliquots

3.4.1.1	Remark	Urine and faeces samples could not be collected completely separated from each other.

4 RESULTS AND DISCUSSION

4.1	**EXCRETION**	
4.1.1	**Radioactivity recovery following gavage with 50 mg/kg bw**	About 75% of the applied dose was excreted in the urine and the remainder of about 25 % in the faeces within 72 hours.

Section A6.2_03

Excretion via urine and faeces in rabbit, following single oral administration by gavage

Annex Point IIA6.2

a) 50 mg/kg, 3 rabbits, $\bar{x} \pm s\bar{x}$

Time after administration (hours)	% of dose $\bar{x} \pm s\bar{x}$		
	Urine	Faeces	Urine + Faeces
0 - 24	57.2 ± 12.4	16.3 ± 6.7	73.5
24 - 48	15.9 ± 9.1	2.9 ± 1.1	18,8
48 - 72	1.0 ± 0.4	3.2 ± 2.4	4.2
0 - 72	74.1 ± 6.8	22.4 ± 9.6	96.5

4.1.2 Radioactivity recovery following gavage with 5 mg/kg bw

About 60% of the applied dose was excreted in the urine and about 15% in the faeces within 72 hours.

b) 5 mg/kg, 3 rabbits, $\bar{x} \pm s\bar{x}$

Time after administration (hours)	% of dose $\bar{x} \pm s\bar{x}$		
	Urine	Faeces	Urine+ Faeces
0 - 24	38.5 ± 19.6	11.6 ± 2.9	50.1
24 - 48	21.6 ± 20.8	2.2 ± 0.7	23.8
48 - 72	0.3 ± 0.1	1.7 ± 1.2	2.0
0 - 72	60.4 ± 6.5	15.5 ± 3.4	75.9

4.2 CONCLUSION

Contrary to the rat findings, where the main way of excretion was by the faeces (up to 91% of the initial dose recovered therein) in the rabbit, renal excretion was found to predominate, with about 60 to75% of the radioactivity recovered in the urine of the treated animals. However, relatively poor balances were noticed, which the author considered to be due to the inadequacy of the metabolite cages. As for the rat, excretion in rabbit was rapid, almost occurring within 48 hours following treatment.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

An ADME study with radiolabelled triclosan was conducted in the early seventies, with rats, mice, rabbits and dogs. The study was conducted prior to the implementation of the current testing guidelines and GLP. The present summary refers only to the experiments conducted with rabbits. The excretion via urine and via faeces was examined in rabbits following single oral administration of radiolabeled test item at dose levels of 5 and 50 mg/kg bw, respectively. Radioactivity measurement was principally based on liquid scintillation counting.

5.2 Results and discussion

As for the rat, excretion in rabbit was rapid, almost occurring within 48 hours following treatment. Contrary to the rat findings, where the main way of excretion was by the faeces (up to 91% of the initial dose recovered therein) in the rabbit, renal excretion was found to predominate, with about 60 to75% of the radioactivity recovered in the urine of the treated animals.

5.3 Conclusion

Section A6.2_03

Excretion via urine and faeces in rabbit, following single oral administration by gavage

Annex Point IIA6.2

5.3.1	Reliability	The study was conducted in the early seventies, i.e. prior to the implementation of current testing guidelines and GLP. The study report shows some deficiencies when comparing to current quality criteria. Nevertheless the results obtained from the rabbit experiments are scientifically acceptable, allowing a comprehensive insight with respect to the excretion of triclosan in rabbit following oral uptake. Therefore, a RL 2 is given.
5.3.2	Deficiencies	- Due to the inadequacy of the metabolite cages, relatively poor balances were noticed. - Urine and faeces samples could not be properly collected separately.

Evaluation by Competent Authorities	
Date	2013-11
Materials and Methods	Agree with applicant´s summary
Results and discussion	Agree with applicant´s conclusion
Conclusion	Agree with applicant´s conclusion
Reliability	2
Acceptability	acceptable
Remarks	

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

Official use only

1 REFERENCE

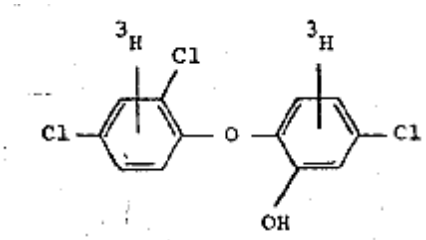
1.1	**Reference**	[redacted] (1972) Study of pharmacokinetics and metabolism in mouse, rat, rabbit and dog. Pharma Research, Pharmacological Chemistry, [redacted] Unpublished Report No. 33/1972, 1st Dec 1972, G, BPD ID A6.02_03
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since supplier is study owner
1.2.3	Criteria for data protection	Data on new [a.s. / b.p.] for [first entry to Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No; the study was conducted in the early seventies, i.e. prior to the implementation of currently accepted testing guidelines.
2.2	**GLP**	No; GLP was not compulsory at the time the study was conducted.
2.3	**Deviations**	Not applicable.

3 MATERIALS AND METHODS

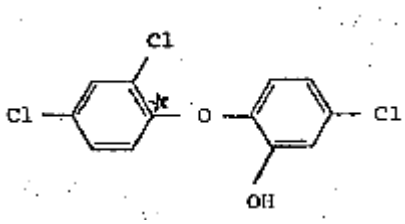
3.1	**Test material**	Triclosan
3.1.1	Tritiated Triclosan	
3.1.1.1	Radiolabelling	Tritiated



3.1.1.2	Specific activity	62.7 µCi/mg
3.1.1.3	Purity	99% chemically and radiochemically pure (isotope dilution analysis and thin layer chromatography)
3.1.1.4	Stability	Proven by testing in neutral, acid and alkaline media; ^{3}H-exchangeability was < 1%.
3.1.2	^{14}C-labelled Triclosan	

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

3.1.2.1	Radiolabelling	^{14}C 
3.1.2.2	Specific activity	13.25 µCi/mg
3.1.2.3	Purity	99.5% chemically and radiochemically pure (thin layer chromatography)
3.1.2.4	Stability	No data
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar WU rats and Sprague-Dawley SIV-50 rats
3.2.3	Source	Not specified
3.2.4	Sex	male and female
3.2.5	Age/weight at study initiation	200 – 250 g
3.2.6	Number of animals per group	4 to 5 animals
3.2.7	Control animals	No
3.2.8	Holding conditions	In metabolism cages; water available ad libitum; food withdrawn 15 hours before test initiation and given back after 2 hours following test item administration.
3.3	**Administration/ Exposure**	
3.3.1		**Oral**

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

3.3.1.1 Application: Gavage

3.3.1.2 Doses: 0.4 and 5 mg/kg bw

3.3.1.3 Vehicle: Ethanol/water (1:9)

3.3.1.4 Sampling

Samples	Dose	Time points or intervals (minutes and hours, post-admin.)	Aim
Blood (N = 4; sex ?; cardiac puncture)	5 mg/kg bw (^{3}H-labelled TS)	30 min, 1, 2, 4, 8, 24 h	total radioactivity count expressed in µg/mL of blood
Urine (N = 4/sex; SIV-50 strain)	0.4 mg/kg bw (^{3}H-labelled TS)	Males: 0-24 h; 24-48 h; 48-72 h; 72-96 h Female: 0-24 h; 24-48 h; 48-2 h; 72-96 h; 96-120 h; 120-144 h; 144-168 h	excretion balance
Feces (N = 4/sex; SIV-50 strain)	0.4 mg/kg bw (^{3}H-labelled TS)	Males: 0-24 h; 24-48 h; 48-72 h; 72-96 h Female: 0-24 h; 24-48 h; 48-2 h; 72-96 h; 96-120 h; 120-144 h; 144-168 h	
Urine (N = 3 males; Wistar WU strain)	5 mg/kg bw (^{3}H-labelled TS)	0-4 h; 4-8 h; 8-24 h; 24-48h; 48-72 h	
Feces (N = 3 males; Wistar WU strain)	5 mg/kg bw (^{3}H-labelled TS)	0-24 h; 24-48 h; 48-72 h	
Organs*, GIT content, carcasse (N = 2 males at 2, 8 and 24 h; N = 3 males at 4 h; Wistar WU strain)	5 mg/kg bw (^{3}H-labelled TS)	2, 4, 8, 24 h	total radioactivity count in tissues expressed in µg/mL and as % of dose applied
Bile (N = 5 males; Wistar WU strain)	5 mg/kg bw (^{3}H-labelled TS)	0-1 h; 0-2 h; 0-4 h; 0-6 h; 0-10 h	Test substance elimination via bile; total radioactivity count expressed as % of dose
			Metabolites identification in pooled bile
*; blood, heart, muscle, brain, bone marrow, testis, lung, adrenals, spleen, thyroid, salivary gland, lymph node, liver, kidney, stomach, intestines			

3.3.2 Intravenously

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

3.3.2.1 Application: injection

3.3.2.2 Dose: 5 mg/kg bw

3.3.2.3 Vehicle: Sodium salt in aqueous solution

3.3.2.4 Sampling

Samples	Dose	Time points or intervals (minutes and hours, post-admin.)	Aim
Blood (N = 5; cardiac puncture)	5 mg/kg bw (^{3}H-labelled TS)	15 and 30 min, 1, 2, 4, 8, 24 h	total radioactivity count expressed in µg/mL of blood
Organs*, GIT content, carcasse (N = 4 males at 2, 8 and 24 h; Sprague-Dawley)	5 mg/kg bw (^{14}C-labelled TS)	2, 4, 8, 24 h	total radioactivity count in tissues expressed in µg/mL and as % of dose applied
*; blood, plasma, heart, aorta, muscle, brain, bone marrow, fat (brown and white), testis, skin, lung, adrenals, spleen, thymus, nerve, thyroid, salivary gland, pancreas, lymph node, liver, kidney			

3.3.3 Intraduodenally

3.3.4 Application: injection

3.3.5 Dose: 5 mg/kg bw

3.3.6 Vehicle: Sodium salt in aqueous solution

3.3.7 Sampling

Samples	Dose	Time points or intervals (hours, post-admin.)	Aim
Bile (N=3 males; Wistar WU strain)	5 mg/kg bw (^{3}H-labelled TS)	0.5, 1, 2, 4, 8, 24 h	Test substance elimination via bile; total radioactivity count expressed as % of dose
			Metabolites identification in pooled bile; total radioactivity count expressed as % of dose

3.4 Analytical methods

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

3.4.1 Radioactivity measurement

Liquid scintillation counting (Packard Model 4000 and 3380 liquid scintillation counters) and thin layer scanning (Berthold Radioactivity Scanner or a Packard "Actigraph").

Preparation of samples for analysis:

Sample	Preparation prior to/for measurement
Urine	none; directly measured
Bile	bleaching (H_2O_2) and dissolving in hyamine hydroxyde
Organs	homogenization, aliquots dissolved in hyamine hydroxide (45°C over 15 hrs) or combustion of aliquot parts up to 100 mg (according to Kalberer and Rutschmann, Helv Chim Acta 44:1956, 1961)
Carcasse	dissolved in 1N NaOH at 70 °C, addition of emulsifier, aliquots of 1 mL for counting
Feces and GIT contents	homogenization, freeze-drying, combustion of aliquots
Blood	0.5 mL samples dried on filter tablets (2 x 2 cm), combustion

3.4.2 Metabolites identification

3.4.2.1 Urine, bile and faecal extracts

Urine, bile and faecal extracts were adjusted to a pH of 1-2 with HCl and extracted several times with petroleum ether. The extracts were identified by thin layer chromatography and compared with authentic material.

3.4.2.2 Glucuronide content in urine

The glucuronide content of the urine was determined by incubation of the urine for 24 hours at 37 °C, pH 6.8, with beta-glucuronidase. After acidification, the aglycone was extracted and further processed as described above (3.4.2.1).

3.4.2.3 Extraction of faeces

Aliquots of the freeze-dried faeces were vigorously shaken in methanol, methanol/water, water and acetone successively; the concentrated extracts were then further processed (see 3.4.2.1)

3.4.2.4 Remark

Owing to the fact, that only little excretion via urine was observed in the study, (<17%), urinary metabolites were consequently not identified.

Instead of the faeces, the bile was preferred for metabolites identification.

4 RESULTS AND DISCUSSION

4.1 EXCRETION

4.1.1 After oral administration

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

4.1.1.1 Blood concentration

The concentration of the test item measured in the blood of rats treated orally with 5 mg/kg bw was 4 µg/mL after 30 minutes. Thereafter, slow decline was observed, with 1.3 µg/mL measured after 24 hours. Between 1and 8 hours after treatment, the concentration curve was found to flatten off to some extent (see values below).

Concentration of radioactivity recovered in the blood of rats treated orally with 5 mg/kg bw radiolabeled test item (µg/mL of blood)	
After (time)	Sex was not specified (mean of 4 animals ± SD)
30 min	4.0±0.8
1 hour	3.1±0.6
2 hours	2.3±0.5
4 hours	2.0±0.2
8 hours	3.2±0.7
24 hours	1.3±0.3

4.1.1.2 Recovery in the bile

After intraduodenal administration of 5 mg/kg bw of test item, about 67% of the initial dose was recovered in the bile within 7-10 hours.

Radioactivity recovery in the bile of rats after intraduodenal injection of 5 mg/kg bw radiolabeled test item (% of dose)	
Time * (h)	Males (mean of 3 animals ± SD)
0-1	16.1±5.2
0-2	36.3±3.3
0-4	54.8±6.0
0-6	62.3±4.5
0-7.5	67.0±3.5
*; Time after treatment (hours)	

After oral administration of 5 mg/kg bw of test item, about 62% of the initial dose was recovered in the bile within 7-10 hours.

Radioactivity recovery in the bile of rats after oral administration of 5 mg/kg bw radiolabeled test item (% of dose)	
Time * (h)	Males (mean of 5 animals ± SD)
0-1	9.1±1.0
0-2	17.5±2.4
0-4	34.4±4.4
0-6	48.2±5.6
0-10	62.5±5.0
*; Time after treatment (hours)	

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

4.1.1.3 Recovery in urine

Test dose 0.4 mg/kg bw:

Total radioactivity recovery in urine sampled over 96 h from male rats treated orally with 0.4 mg/kg bw of test item was about 3%. Therefrom, about 90% already were recovered in the urine sampled during the first 24 hours post treatment. Total radioactivity recovery in urine sampled over 168 h from female rats treated orally with 0.4 mg/kg bw of test item was about 17%. Therefrom, about 90% already were recovered in the urine sampled during the first 24 hours post treatment. Thus, the parent compound (and/or its metabolite(s)) was rapidly excreted via urine.

Radioactivity recovery in the urine of rats treated orally with 0.4 mg/kg bw radiolabeled test item (% of dose)		
Time *	Males (mean of 4 animals ± SD)	Females (mean of 4 animals ± SD)
0-24	1.7±0.3	14.1±1.8
24-48	0.9±0.2	1.9±0.7
48-72	0.4±0.08	0.7±0.3
72-96	0.3±0.06	0.2±0.05
96-120	-	0.1±0
120-144	-	<0.05
144-168	-	0.1
0-168	3.3%	17.1%
*; Time after treatment (hours)		

Test dose 5 mg/kg bw:

Total radioactivity recovery in urine sampled over 72 h from male rats treated orally with 5 mg/kg bw of test item was 3%. Therefrom, about 90% already were recovered in the urine sampled during the first 24 hours post treatment. Thus, the parent compound (and/or its metabolite(s)) was rapidly excreted via urine.

Radioactivity recovery in the urine of rats treated orally with 5 mg/kg bw radiolabeled test item (% of dose)	
Time * (h)	Males (mean of 3 animals ± SD)
0-4	0.1±0.07
4-8	0.6±0.4
8-24	1.3±0.2
24-48	0.6±0.1
48-72	0.4±0.1
0-72	3%
*; Time after treatment (hours)	

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

4.1.1.4 Recovery in faeces

Test dose 0.4 mg/kg bw:

Total radioactivity recovery in faeces sampled over 96 h from male rats treated orally with 0.4 mg/kg bw of test item was about 78%. Therefrom, about 70% already were recovered in the faeces sampled during the first 24 hours post treatment. Total radioactivity recovery in faeces sampled over 168 h from female rats treated orally with 0.4 mg/kg bw of test item was about 68%. Therefrom, about 60% already were recovered in the faeces sampled during the first 24 hours post treatment. Thus, the parent compound (and/or its metabolite(s)) was rapidly excreted via faeces.

Radioactivity recovery in the faeces of rats treated orally with 0.4 mg/kg bw radiolabeled test item (% of dose)		
Time *	Males (mean of 4 animals ± SD)	Females (mean of 4 animals ± SD)
0-24	52.1±11.8	42.9±3.8
24-48	22.1±7.9	18.8±7.1
48-72	3.0±1.2	4.7±1.2
72-96	0.8±0.3	1.2±0.3
96-120	-	0.3±0.07
120-144	-	0.1
144-168	-	0.1
0-168	78%	68.1%
*; Time after treatment (hours)		

Test dose 5 mg/kg bw:

Total radioactivity recovery in faeces sampled over 72 h from male rats treated orally with 5 mg/kg bw of test item was 91%. Therefrom, about 90% already were recovered in the faeces sampled during the first 24 hours post treatment. Thus, the parent compound (and/or its metabolite(s)) was rapidly excreted via faeces.

Radioactivity recovery in the faeces of rats treated orally with 5 mg/kg bw radiolabeled test item (% of dose)	
Time * (h)	Males (mean of 3 animals ± SD)
0-24	87.4±1.5
24-48	3.8±1.9
48-72	0.1±0.07
0-72	91.3%
*; Time after treatment (hours)	

4.1.2 After intravenous injection

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

4.1.2.1 Blood concentration

The concentration of the test item measured in the blood of rats after iv injection of 5 mg/kg bw was 5.4 µg/mL after 10 minutes and increased to 6 µg/mL after 30 minutes. According to the author of the study, the increase was presumed to be due to the rapid formation of highly polar metabolites with a smaller distribution volume. Thereafter, slow decline was observed, with 0.7 µg/mL measured after 24 hours. Between 1and 8 hours after treatment, the concentration curve was found to flatten off to some extent (see values below).

Concentration of radioactivity recovered in the blood of rats treated by iv injection with 5 mg/kg bw radiolabeled test item (µg/mL of blood)

After (time)	Sex was not specified (mean of 5 animals ± SD)
10 min	5.4±0.3
30 min	6.0±0.2
1 hour	5.6±0.6
2 hours	4.9±0.4
4 hours	4.4±0.5
8 hours	3.3±0.4
24 hours	0.7±0.2

4.2 ABSORPTION

For determination of the absorption, the total surface areas under the blood concentration curves obtained after oral and intravenous treatment with the radiolabelled test item were compared. The comparison revealed that the test item was absorbed to about 70 to 80%. This was further supported by the high rate of biliary excretion, as shown in 4.1.1.2.

4.3 DISTRIBUTION IN ORGANS

4.3.1 After oral administration

The concentration of radiolabeled test item recovery in the organs/tissues following oral administration of 5 mg/kg bw were reported to be relatively low. After 2 hours following treatment, the highest amounts of radioactivity were recovered in the gastrointestinal tract (about 67 µg/g fresh tissue), the liver (5.2 µg/g fresh tissue) and kidneys (3.2 µg/g fresh tissue). After 4 hours, the highest amounts of radioactivity were recovered in the gastrointestinal tract (about 25 µg/g fresh tissue), the liver (5.1 µg/g fresh tissue), and kidneys (3.4 µg/g fresh tissue). A gradual decline with time was noticed, and after 24 hours, the amounts detected in the organs/tissues did no more exceed 0.6 µg/g fresh tissue.

4.3.2 After IV injection

After 1 hour following treatment, the highest amounts of radioactivity were recovered in the brown fat (6.9 µg/g fresh tissue), the liver (6.1 µg/g fresh tissue), and the lungs (5.5 µg/g fresh tissue), followed by the kidneys (3.8 µg/g fresh tissue). After 6 hours, only 1 µg/g fresh tissue remained in the brown fat, indicating rapid elimination from there; in liver, kidney and lungs, 3.4, 2.6 and 1.9 µg/g fresh tissue were recovered, respectively.

4.4 TOTAL BALANCE

In terms of balance, total recovery was 100% of the initially orally administered dose of radioactivity; for details, see the following table (issue from study report):

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

Distribution and balance in the rat after oral administration of 5 mg/kg bw of radiolabeled test item expressed as % of dose:

$\bar{X}$, 2 rats (4 hr. values for 3 rats)

Organ	% of dose			
	2 hrs.	4 hrs.	6 hrs.	8 hrs.
Blood	2.79	1.23	0.97	0.11
Heart	0.03	0.03	0.02	0.01
Muscle	0.06	0.08	0.06	0.05
Brain	0.03	0.04	0.04	0.03
Bone marrow	0.008	0.007	0.002	0.004
Testis	0.13	0.15	0.13	0.07
Lung	0.24	0.19	0.15	0.04
Adrenal	0.008	0.009	0.004	0.005
Spleen	0.01	0.02	0.005	0.005
Thyroid	0.001	0.001	0.002	0
Liver	3.66	3.44	2.24	0.34
Kidneys	0.54	0.52	0.26	0.10
Stomach	7.24	2.15	0.09	0.02
Intestine	3.77	4.06	1.71	0.14
Urine	0.21	0.47	0.60	2.44
Gastro-intestinal contents	71.91	76.32	76.60	5.17
Faeces	-	-	13.42	89.70
Carcase	13.85	8.93	9.54	2.84
T o t a l	104.53	99.67	105.87	101.10

It was noticed that up to 8 hours after administration, about 75% of the initial dose was found in the combined contents of the stomach and intestine; it was presumed that this was due to the enterohepatic circulation of the test item.

4.5 METABOLITES See remark 3.4.2.4

4.5.1 Identification in bile

About 60% of the radiolabeled material present in the bile, i.e., about 40% of the initial radioactive dose could be extracted. Half of the extract could be identified by isotope dilution analyses as unchanged parent compound.

Thin layer chromatography revealed the presence in the extract of at least 2 or 3metabolites, which, on the basis of their Rf values, were highly polar. The remaining radioactivity in the bile could be extracted by after splitting with beta-glucuronidase. Mainly unchanged parent compound was identified in the thin layer chromatogram, with 20-30% of the radioactivity detected in the bile originating from the glucuronide metabolite of the parent compound.

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

4.6 CONCLUSION

The main findings of the present study can be summarized as follows:

The comparison of the total surface areas under the blood concentration curves obtained after oral and intravenous administration indicates an absorption level of about 70 to 80% of the test item which is further supported by the high rate of biliary excretion. Following oral uptake in rat, in terms of balance, total recovery is 100% of the initial dose. Rapid elimination occurs, almost within the first 24 hours, with up to 90% of the initial dose being excreted via the faeces; excretion via urine is comparatively low (<20%). A high rate of biliary elimination was demonstrated, and a plateau in the blood concentration curve was observed, both indicating that the substance undergoes enterohepatic circulation. The intravenous application experiment first revealed an increase in the concentration of test item in blood (within 10 to 30 minutes), which was then followed by slow subsequent decline of the blood concentration. The increase was attributed to the rapid formation of highly polar metabolites with a smaller distribution volume; the decrease was attributed to the marked enterohepatic circulation of the test item. Only low amounts of radioactivity were recovered in the organs and tissues for a relatively short time, indicating that no accumulation in organs/tissues occurs. Following oral uptake, the highest amounts were measured in the biotransformation organs, liver and kidneys two hours after treatment. With respect to metabolite identification, in the rat bile, where about 65% of the dose initially given was eliminated, about 30% of the radioactivity measured referred to the unchanged parent compound; approximately the same percentage was due to unconjugated, more polar metabolites (as demonstrated by T.L.C.), and the remaining third to glucuronides.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

An ADME study with radiolabelled triclosan was conducted in the early seventies, with rats, mice, rabbits and dogs. The study was conducted prior to the implementation of the current testing guidelines and GLP. The present summary refers only to the experiments conducted with rats. Several experiments were conducted, with the rats receiving radiolabeled test item either gavage (0.4 and 5 mg/kg bw), by intravenous injection (5 mg/kg bw) or by intraduodenal injection (5 mg/kg bw). On the basis of these experiments, the absorption, distribution, metabolism, and excretion of triclosan in rats was investigated. Radioactivity measurement and metabolite identification were principally based on liquid scintillation counting and thin layer chromatography.

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

5.2	**Results and discussion**	Based on results of the present study, following main findings can be retained: In rat, triclosan is well absorbed following oral uptake, since an absorption level of 70 to 80% could be showed. A high rate of biliary elimination occurs, indicating that triclosan undergoes enterohepatic circulation. The elimination is rapid, and the main way of excretion is via the faeces (up to 90% of the initial dose); excretion via urine is comparatively low. No accumulation in organs/tissues occurs; the highest amounts were measured in the biotransformation organs, liver and kidneys two hours after treatment (about 5 µg/g fresh tissue); after 24 hours, the detected amounts did not exceed 0.6 µg/g fresh tissue. With respect to metabolite identification, in the rat bile, where about 65% of the dose initially given was eliminated, about 30% of the radioactivity measured referred to the unchanged parent compound; approximately the same percentage was due to unconjugated, more polar metabolites (as demonstrated by T.L.C.), and the remaining third to glucuronides.
5.3	**Conclusion**	
5.3.1	Reliability	The study was conducted in the early seventies, i.e. prior to the implementation of current testing guidelines and GLP. The study report shows some deficiencies when comparing to current quality criteria. Nevertheless, the methods which were applied are considered suitable, and the results of the different experiments taken together are scientifically acceptable, allowing a comprehensive insight with respect to the fate of triclosan in rat following oral uptake. Therefore, a RL 2 is given.
5.3.2	Deficiencies	- Tritiated and ^{14}C-labelled test item were used; however, in the method part of the study report, it was not specified why. - The description of substance preparation for iv and intraduodenal injection was poor. - No description on intraduodenal injection procedure was given. - The description of the study design in the method part of the study report was poor; for some experiments, it was not recognizable whether animals of both sexes or not were used. As example, in the experiment on blood concentration, see 3.3.1.4. - Different rat strains were used, but without giving any explanation why (see part E of the study report); strain designation in part E and in the result tables were in part different.

Section A6.2_03 Pharmacokinetics and metabolism in rat

Annex Point IIA6.2

Evaluation by Competent Authorities	
Date	2013-11
Materials and Methods	Agree with applicant´s summary
Results and discussion	Agree with applicant´s conclusion
Conclusion	Agree with applicant´s conclusion
Reliability	2
Acceptability	acceptable
Remarks	

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

Official use only

1 REFERENCE

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

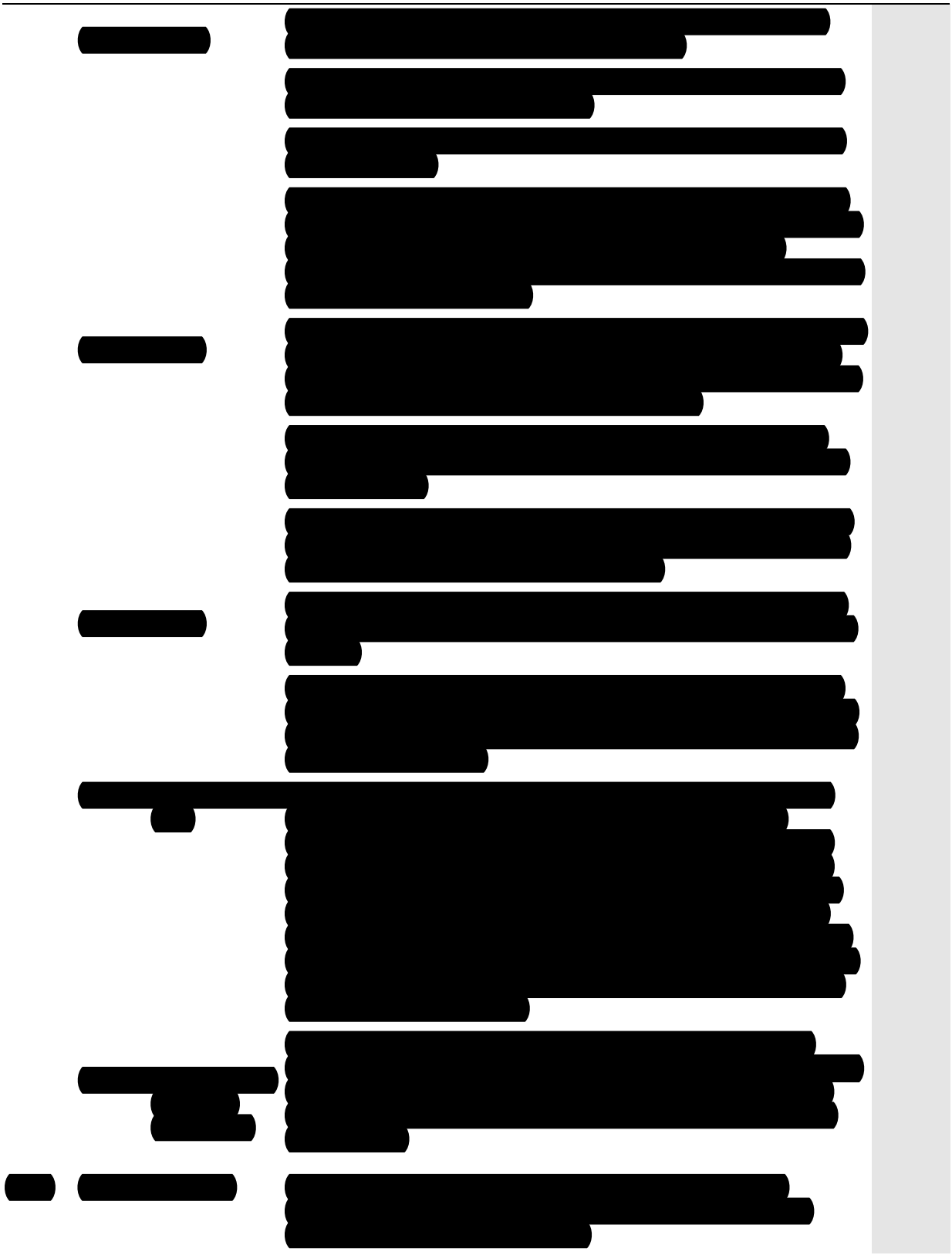
Annex Point IIA6.2

[redacted]

3.3.6 [redacted]

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2



Section A6.14_01 Annex Point IIA6.2	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)

3.3.8 [redacted]

Section A6.14_01 Annex Point IIA6.2	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)

Section A6.14_01 Annex Point IIA6.2	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)

[redacted]

Section A6.14_01	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)
Annex Point IIA6.2	

[redacted]

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

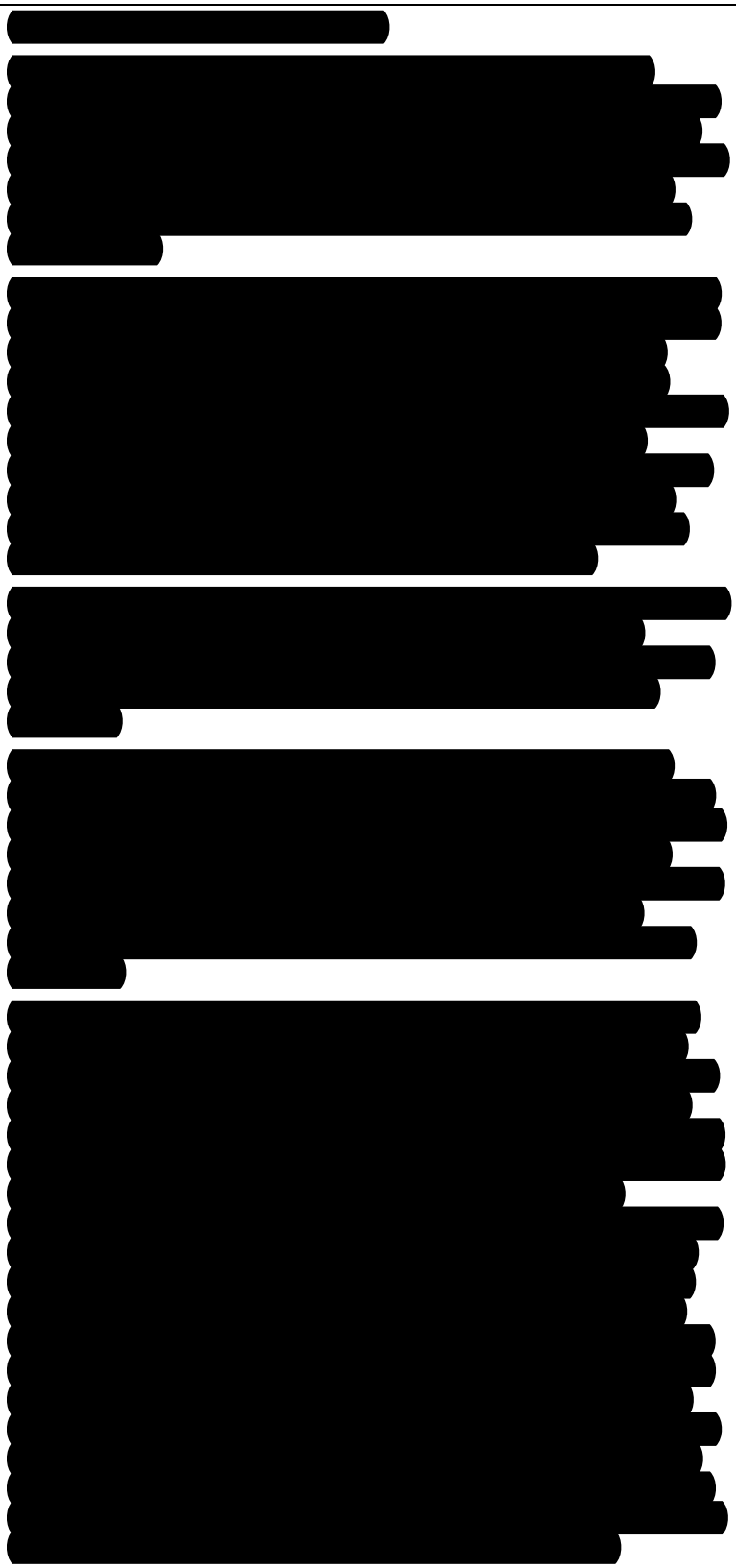
Annex Point IIA6.2

[redacted]

Section A6.14_01 Annex Point IIA6.2	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)

[redacted]

Section A6.14_01	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)
Annex Point IIA6.2	



Section A6.14_01	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)
Annex Point IIA6.2	

4.3 [redacted]

Section A6.14_01	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)
Annex Point IIA6.2	

Section A6.14_01 Annex Point IIA6.2	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]

[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]

Section A6.14_01

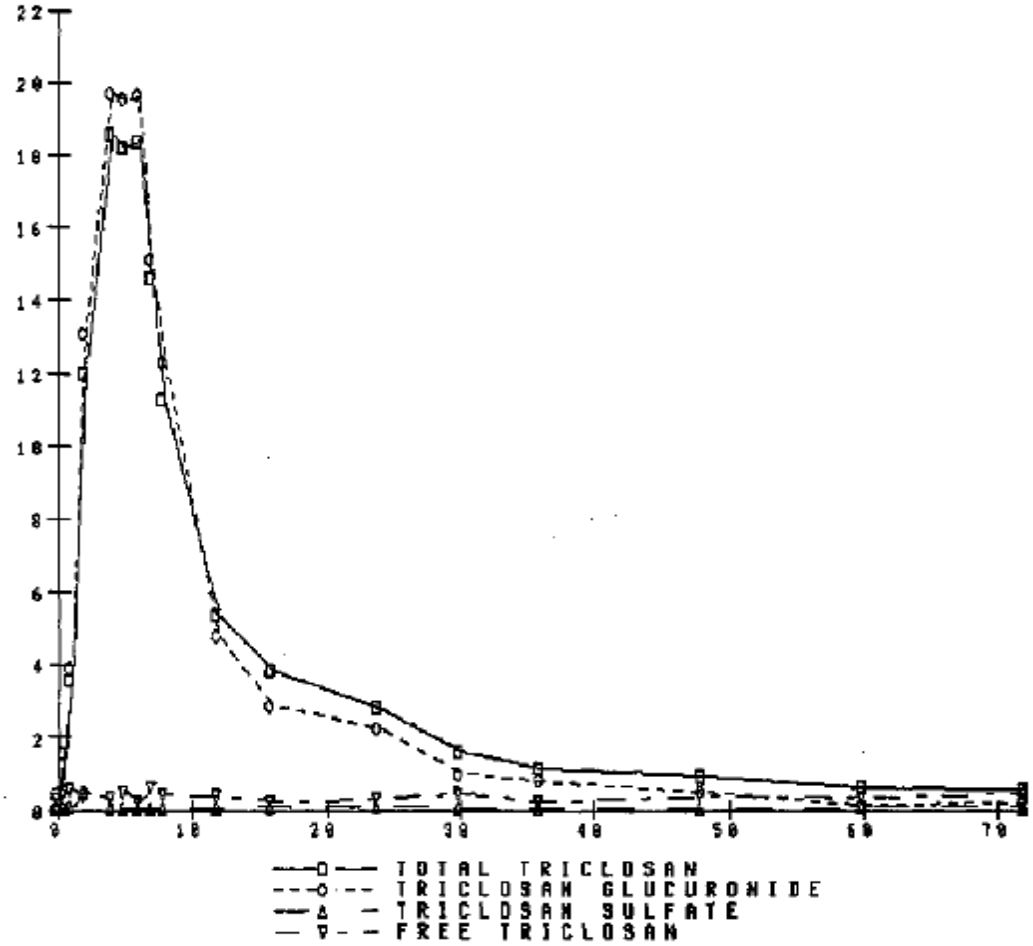
Annex Point IIA6.2

[redacted]

[redacted] by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2012
Materials and Methods	Agree with the applicant´s version.
Results and discussion	Agree with the applicant´s version.
Conclusion	Agree with the applicant´s version.
Reliability	1
Acceptability	Acceptable
Remarks	-

[redacted]

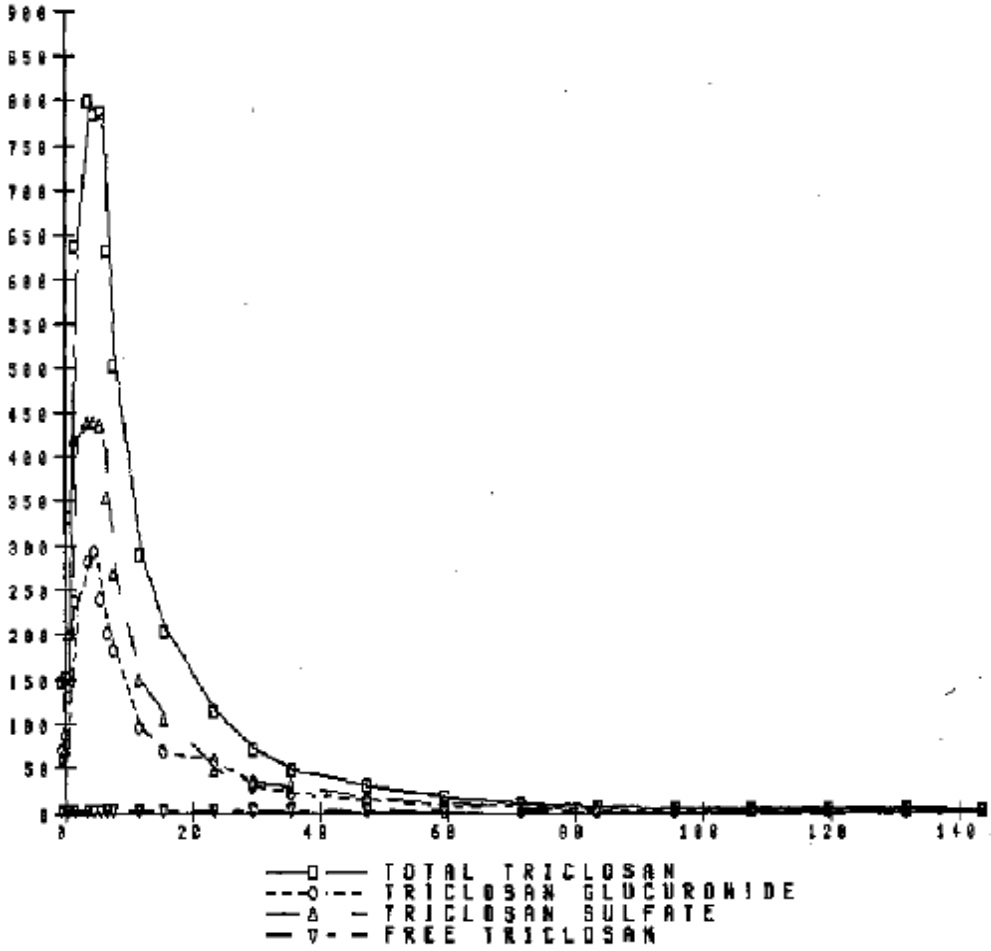
Figure 1. Median concentration-/time courses of total Triclosan, free Triclosan, Triclosan-glucuronide and Triclosan-sulphate following single dose administration respectively:



[redacted]

Section A6.14_01	Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)
Annex Point IIA6.2	

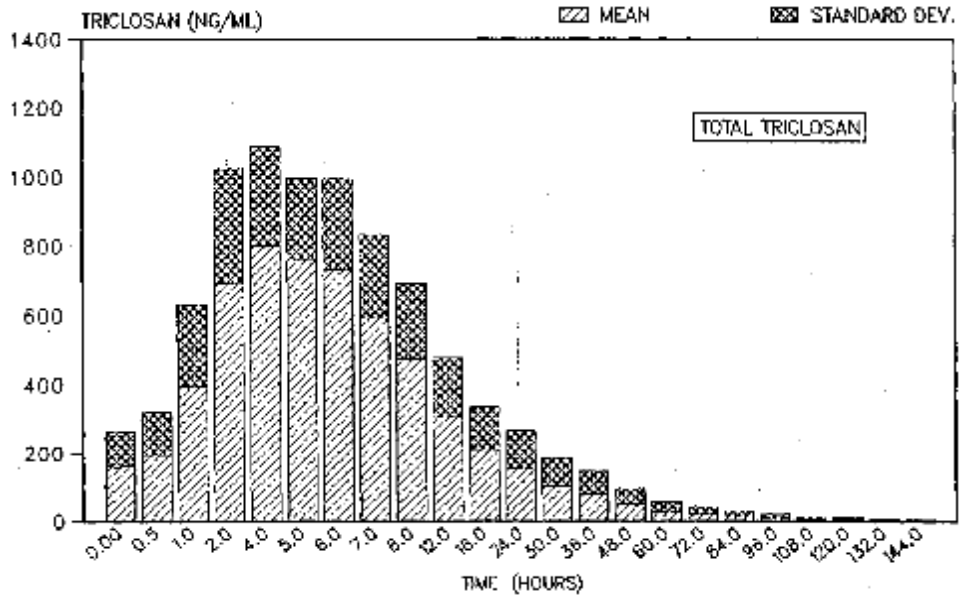
[redacted]



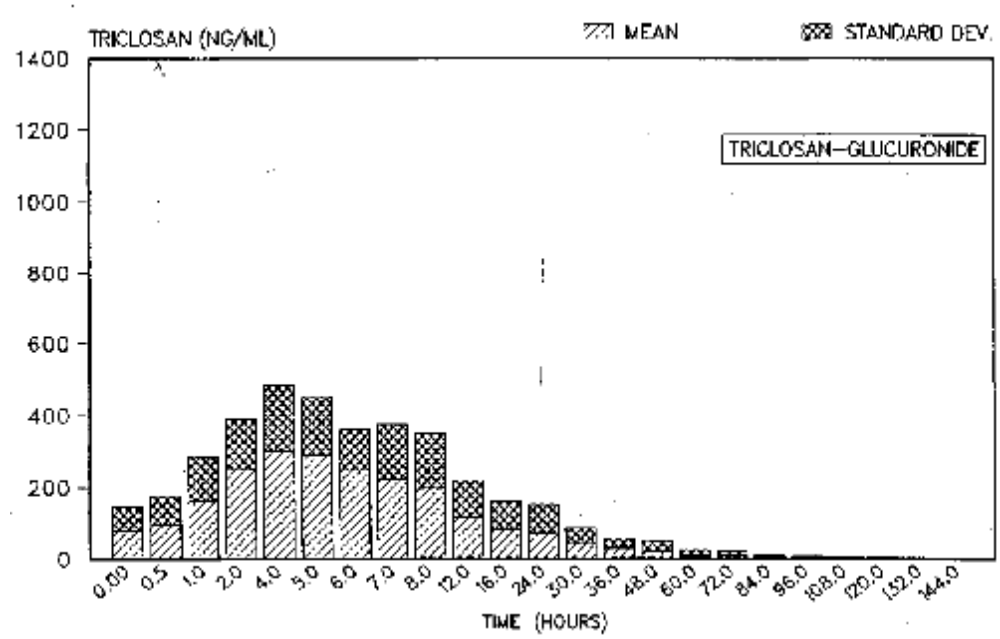
[redacted]

[redacted]

of [redacted]



[redacted]

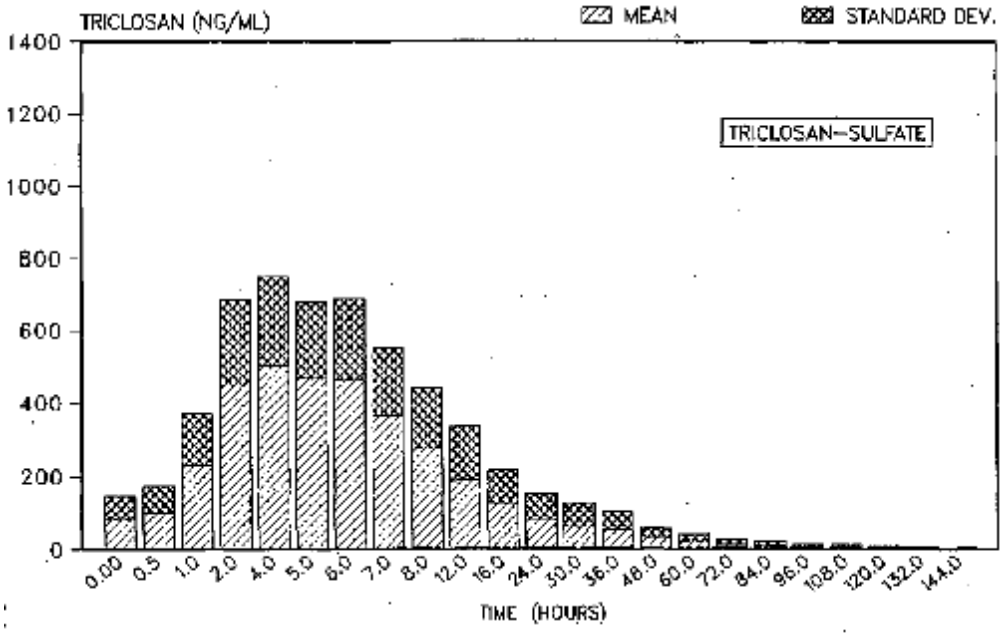


[redacted]

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]



[redacted]

	TCS			
	TOTAL	GLUCURONIDE	SULPHATE	FREE
FIRST ADMINISTRATION (SINGLE DOSE)				
AUC_∞ [ng*h/ml]	185.38 (217.13)	180.57 (222.47)	8.10 (13.21)	13.34 (11.91)
) GLUCURONIDE/ SULPHATE		16.84		
% OF TOTAL TCS	(100)	96.94	4.35	7.16
LAST ADMINISTRATION (STEADY STATE)				
AUC_τ * [ng*h/ml]	567.71 (598.62)	209.25 (235.10)	298.28 (362.35)	1.12 (1.21)
) GLUCURONIDE/ SULPHATE		0.70		
% OF TOTAL TCS	(100)	36.86	52.54	0.20

* Dose-corrected to 1 mg

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]

	ABSORPTION/DISTRIBUTION PHASE		TERMINAL ELIMINATION PHASE		β-ELIMINATION PHASE		α-DISTRIBUTION PHASE	
	$t_{1/2abs}$ [h]	AUC %	$t_{1/2\beta}$ [h]	AUC %	$t_{1/2\beta}$ [h]	AUC %	$t_{1/2\alpha}$ [h]	AUC %
TCS-GLUCURONIDE	1.49	~57.9	18.28	42.7	5.79	39.0	0.28	92.9
	(1.38)	(~85.6)	(19.19)	(58.0)	(7.79)	(61.4)	(0.36)	(113.1)
TCS-SULPHATE	1.34	~53.8	21.22	42.8	8.01	32.1	0.32	88.1
	(1.35)	(~75.3)	(21.83)	(80.9)	(8.37)	(38.5)	(0.13)	(104.0)

[redacted]

	% OF GIVEN DOSE		RENAL ELIMINATION HALFLIFE [h]	
COMPOUND	SINGLE DOSE	STEADY STATE	SINGLE DOSE	STEADY STATE
TOTAL TCS	97.10	121.34	7.39	33.61
	(90.52)	(125.75)	(9.01)	(14.10)
FREE TCS	-------	0.88	-------	------
		(1.52)		
TCS-GLUCURONIDE	89.47	111.52	7.12	12.26
	(85.09)	(116.44)	(7.27)	(13.33)
TCS-SULPHATE	7.26	6.81	11.91	18.27
	(6.43)	(7.77)	(12.66)	(17.55)

Section A6.14_01 **Other test(s) related to the exposure of humans: Safety (Tolerance) and Pharmacokinetics of Triclosan (TCS)**

Annex Point IIA6.2

[redacted]

	ELIMINATION IN THE FECES DURING 8 DAYS IN STEADY STATE	
	AMOUNT [ug]	PERCENT OF DOSE [%]
TOTAL TCS	7.494	8.33
	(9.659)	(10.73)
FREE TCS	4.210	4.68
	(4.981)	(5.53)
TCS-GLUCURONIDE	2.080	2.31
	(2.419)	(2.69)
TCS-SULPHATE	1.423	1.59
	(2.318)	(2.58)

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2001), [redacted] *In Vitro* Absorption through Pig Epidermis. [redacted] Report No. [redacted], date: 2001-03-28 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD Draft Guideline 428 (1996)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Non-labelled test substance was used Only one concentration was used. The recovery was less than 100 ±10%.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] = DCPP	
3.1.1	Non-labelled compound	O/W formulation with [redacted] contains 29.4% DCPP)	
3.1.2	Lot/Batch number	[redacted]	
3.1.3	Specification		
3.1.3.1	Description	White emulsion	
3.1.3.2	Purity	0.3% DCPP (w/v)	x
3.1.3.3	Stability	Unknown	
3.1.3.4	**Labelled compound**	Not used	
3.2	**Test System**		
3.2.1	Species	Pig	
3.2.2	Source	Abattoir	
3.3	**Administration/ Exposure**		
3.3.1	Preparation of test	Ears from adult pigs were obtained from an abattoir. The ears were washed under running water and badly damaged ears rejected. The remaining ears were shaved with animal clippers and whole skin (epidermis + dermis) was separated from the cartilage using a scalpel. The skin samples were immersed in water at 60°C for 55-60 seconds and the epidermis teased away from the dermis. Each epidermal membrane was given an identifying number and stored frozen on aluminium foil. Discs of approximately 3.3 cm diameter of prepared skin membrane	

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

		from at least three subjects were mounted, dermal side down, in diffusion cells held together with individually numbered clamps.	
3.3.2	Determination of membrane integrity	The donor and receptor chambers of the cells were filled with physiological saline (approx 0.9% w/v sodium chloride in water) and placed in a water bath maintained at 32 ± 1°C. The integrity of the membranes was determined by measurement of the electrical resistance across the skin membrane. Membranes with a measured resistance <3.0 kΩ were regarded as having a lower integrity than normal and were not used.	
3.3.3	Concentration of test substance	0.3% DCPP (w/v) 30 µg DCPP/cm² skin	
3.3.4	Volume applied	25.4 µL (10 µL/cm²)	
3.3.5	Size of test site	2.54 cm²	
3.3.6	Exposure period	0.5, 24 h	
3.3.7	Receptor fluid	25% Ethanol in pH 7 phosphate buffered saline	
3.3.8	Sampling time	Receptor fluid: 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 20, and 24 h Tape strips, remaining receptor fluid and washings: at study termination (24 h).	x
3.3.9	Samples	Receptor fluid, tape strips, washings.	
		4 RESULTS AND DISCUSSION	
4.1	**Recovery of labelled compound**	0.5 h exposure: 88.4 ± 9.05% 24 h exposure: 85.9 ± 1.7% see Table A6_2-1	
4.2	**Percutaneous absorption**	0.5 h exposure: Receptor fluid: 6.22%, epidermis: 2.87%, potential absorption: 9.09% 24 h exposure: Receptor fluid: 19.2%, epidermis: 15.2%, potential absorption: 34.4% see Table A6_2-1	x
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The absorption of DCPP from a formulation containing a nominal 0.3% w/v DCPP has been measured *in vitro* through pig epidermis. The formulation was applied undiluted at a rate of 10 µL/cm^2; all applications were occluded for a total exposure period of 24 h. The applications were split into two groups, where for Group 1 the dose remained in continuous contact with the epidermis for 24 h, while for Group 2 the surface of the epidermis was rinsed 0.5 h after application. These applications were designed to simulate potential human dermal contact with the formulation during normal use. Additionally, the distribution of DCPP in the epidermis and within the test system was assessed and the mass balance determined.	
5.2	**Results and discussion**	**Absorption of DCPP** Average absorption rates during the first 0.5 h of exposure were 0.100 and 0.177 µg/cm^2/h for Groups 1 and 2, respectively. The fastest absorption of DCPP for Group 1 was between 0.5-4 h (0.403 µg/cm^2/h), while for Group 2 the maximum rate (0.209 µg/cm^2/h) was between 0.5	

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

- 3 h. During the following period up to 24 h, absorption rates were 0.212 and 0.059 µg/cm^2/h for Groups 1 and 2, respectively. The average rates over the entire 24 h exposure period were 0.253 and 0.076 µg/cm^2/h, respectively.

The proportions of the DCPP dose absorbed at 0.5 h were 0.17% and 0.29% (Groups 1 and 2 respectively). By 4 h, the amounts were 4.82% and 2.42%, respectively and by 24 h 19.2% and 6.22% of the applied dose.

Distribution of DCPP in the epidermis

The proportion of the DCPP dose recovered from the *stratum corneum* was 35.9% from Group 1 and 2.00% from Group 2, with 20.0% and 1.13%, respectively, being found in the first strip. The remaining epidermis contained 15.2% and 2.87% of the dose, respectively. The total amounts in the whole epidermis were 51.1% for Group 1 and 4.87% for Group 2.

Distribution of DCPP within the test system

For Group 1, 51.1% was recovered from complete epidermis and the proportion absorbed was 19.2%. Only 5.68% was washed off the epidermis at 24 h and the amounts recovered from the spreaders and donor chambers totalled 9.87%.

For Group 2, only a total of 4.87% was recovered from the complete epidermis and the proportion absorbed was 6.22%. The amount removed during the decontamination at 0.5 h was 71.5%, with a further 1.01% being washed off at 24 h. The amounts recovered from the donor chambers at 24 h and spreaders totalled 4.71%.

5.3 Conclusion

1. The results obtained in this *in vitro* study indicate that the absorption of DCPP from this O/W formulation through pig epidermis would be regarded as very slow.
2. The majority (up to 70%) of the DCPP dose can be removed from the skin surface by a simple washing procedure after a contact period of 0.5h.
3. After the DCPP dose was washed off the epidermis at 0.5h, there was up to a 3-fold reduction in absorption in the 24h after application.
4. The wash at 0.5 h reduced the amount of DCPP associated with the epidermis by almost 10-fold at 24 h.
5. The majority of the DCPP dose associated the epidermis after 24h continuous contact was located in the *stratum corneum*.
6. The data predict that, under conditions of potential human dermal contact, absorption of DCPP from this O/W formulation would be minimal.
7. The tested formulation contains 85.3% water and the results obtained in this study are therefore representative for other water-based formulations of DCPP.

5.3.1 Reliability: 1

5.3.2 Deficiencies: The recovery was less than 90%. However, the recovery was still >85%, which is deemed acceptable for unlabelled test substance.

Only one concentration (0.3% pure DCPP) was tested. This concentration is close to the anticipated DCPP concentration in the biocidal product (0.2% DCPP). The relevant concentration for the envisaged applications is therefore covered. The use of lower

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

concentrations (in-use dilutions) is also covered by this study. Dermal absorption and substance concentration are inversely correlated when a certain concentration threshold is exceeded. This is because of increasingly saturated transport processes in the epidermis. However, 0.3% is thought to be a sufficiently low concentration at which dermal penetration is not yet limited.

In addition, *in-vitro* studies of dermal absorption tend to yield significantly higher dermal penetration percentages than *in-vivo* studies, especially if skin-bound residues are considered as potentially absorbable. The exclusive use of *in-vitro* data for assessment of human *in-vivo* exposure is inherently conservative. Therefore, the use of 9.1% for rinse-off products and 34.4% for all-day exposures is deemed suitable for assessing the entire concentration range that users can be exposed to.

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date June 2010

Materials and Methods

3.1.3.2: *Replace:* >99% w/v, Batch: [redacted]

3.3.8: *Amend:* The upper layer of the epidermis were removed by repeated application of up to 5 tape strips.

Results and discussion

4.2: *Addition:* The material removed from by the 2 first tape strips (layer of the stratum corneum) is regarded as unabsorbed.

Conclusion

5.3: *Remark:* The OECD Guidance document for the conduct of skin absorption studies (No 28) recommends 15-25 tape strips to take off the total stratum corneum. In this study only 5 tape strips were used, consequently the detached amount should not represent the total but only the superficial stratum corneum. However in the Manual of Technical Agreements of the Biocides TMs (MOTA) it was recommended that usually only the first 2 tape strips are considered as not-absorbed.

Consequently dermal absorption rates are concluded as follows:

1) 0.5h exposure:
Receptor fluid: 6,2%
Epidermis: 2.9%
The last 3 of 5 tape strips: 0.7% *

Potential absorption: 9,8%

2) 24h exposure:
Receptor fluid: 19.2%
Epidermis: 15.2%
The last 3 of 5 tape strips: 9.8% *

Potential absorption: 44,2%

In case flux estimates are used for dermal exposure calculations, the following values are proposed:

Mean flux to receptor fluid over 0.5 hours continuous exposure: 0.177 µg/cm2 *h

Mean flux to receptor fluid over 24 hours continuous exposure: 0.253 µg/cm^{2} *h

Flux into skin (epidermis and stratum corneum strip 3-5) over 30 minutes exposure, measured 23.5 hours post exposure: 1.08 µg/cm^{2} *0.5h

Flux into skin (epidermis and stratum corneum strip 3-5) over 24 hours continuous exposure: 4.8 µg/cm^{2} *24h

Reliability 2

Acceptability Acceptable

Remarks No results from a reference substance or historical data for a number of reference substances that show the performance and reliability of the test system in the performing laboratory were submitted.
Table 6_1-2 below was added by the RMS.

Section A6.2 Percutaneous absorption (*in vitro* test)

Annex Point IIA6.2 6.2 *In vitro* dermal absorption study through pig epidermis

Table A6_2-1. Table for *in vitro* percutaneous absorption study in pig skin (mean of 5-6 values per group)

Samples	Percent of Applied Dose	
	0.5 h exposure	**24 h exposure**
1. Spreaders	4.29	4.41
2. Donor chamber	0.42	5.46
3. Skin wash 30 min	71.5	–
4. Skin wash 24 h	1.01	5.68
5. Stratum Corneum (strip 1)	1.13	20.0
6. Stratum Corneum (strip 2)	0.14	6.07
7. Stratum Corneum (strip 3)	0.10	5.24
8. Stratum Corneum (strip 4)	0.45	2.50
9. Stratum Corneum (strip 5)	0.18	2.06
10. Stratum Corneum (total strips)	2.00	35.9
11. Epidermis	2.87	15.2
12. Receptor Fluid	6.22	19.2
13. Potentially Absorbable (11+12)	**9.09**	**34.4**
14. Total recovery	**88.4**	**85.9**

Table A6_1-2 Information on flux into the receptor fluid

1. DETAILS OF APPLICATION OF TEST MATERIALS	2. MEAN ABSORPTION RATES		3. MEAN AMOUNT AND PERCENT OF DOSE ABSORBED		
	Time period (h)	Absorption rate ($\mu g/cm^2/h \pm SEM$)	Time (h)	Amount ($\mu g/cm^2$)	Percent absorbed
Group 1: 24h continuous contact			0.5	0.05	0.17
	0-0.5	0.100 ± 0.030	1	0.21	0.70
Undiluted O/W emulsion			2	0.61	2.03
	0.5-4	0.403 ± 0.052	3	1.04	3.45
(Nominally 0.3% FAT 80'220/B)			4	1.45	4.82
	4-24	0.212 ± 0.017	6	2.14	7.12
10µl/cm² (nominally 30µg FAT 80'220/B /cm²)			8	2.74	9.13
	0-24	0.253 ± 0.024	10	3.28	10.9
Occluded			12	3.77	12.6
			16	4.53	15.1
n = 6			20	5.19	17.3
			24	5.76	19.2

1. DETAILS OF APPLICATION OF TEST MATERIALS
Group 2: 0.5h contact (24h measurement)
Undiluted O/W emulsion
Nominally 0.3% FAT 80'220/B
10µl/cm² (nominally 30µg FAT 80'220/B /cm²)
Occluded
n = 5

2. MEAN ABSORPTION RATES	
Time period (h)	Absorption rate (µg/cm²/h ± SEM)
0-0.5	0.177 ± 0.057
0.5-3	0.209 ± 0.031
3-24	0.059 ± 0.012
0-24	0.076 ± 0.012

3. MEAN AMOUNT AND PERCENT OF DOSE ABSORBED		
Time (h)	Amount (µg/cm²)	Percent absorbed
0.5	0.09	0.29
1	0.22	0.72
2	0.43	1.43
3	0.61	2.04
4	0.73	2.42
6	0.90	3.00
8	1.07	3.57
10	1.22	4.06
12	1.35	4.48
16	1.55	5.17
20	1.71	5.71
24	1.87	6.22

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](1999), [redacted] 28-Day Oral Toxicity (Gavage) Study in the Wistar Rat. [redacted] Project No. 712135, date: 1999-04-13 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD Guideline 407 (1995) = EC Method B.7 (1996)
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in Section 2 of dossier.
3.1.2.1	Description	Solid powder
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stable under storage conditions Stability in vehicle was confirmed.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	HanIbm:WIST (SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ + ♀
3.2.5	Age/weight at study initiation	Age: 6 weeks (at delivery) Weight range: 140-157 g (♂) and 111-132 g (♀).
3.2.6	Number of animals per group	Control & high-dose group: 10 per sex Low- and mid-dose group: 5 per sex
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	Once daily
3.3.3	Post-exposure period	14 day for recovery group (control + high-dose animals only)

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

3.3.4	**Oral**	
3.3.4.1	Type	Gavage
3.3.4.2	Dose	50, 150, 750 mg/kg bw/day
3.3.4.3	Vehicle	PEG 300
3.3.4.4	Concentration in vehicle	10, 30, 150 mg/mL
3.3.4.5	Dose volume	5 mL/kg bw
3.3.4.6	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	General cageside observations (daily): The animals were observed for clinical signs once before commencement of administration; twice daily on days 1-3 (target: approximately 1 and 3 hours after administration); as well as once daily on days 4-28 (target: approximately 1 hour after administration), and once daily during days 29-42 (recovery). Detailed clinical observations (weekly): The animals were observed in their home cages, outside their home cages in a standard arena and in the hand. These observations were performed in random sequence once before commencement of administration and once weekly (weeks 1-3) thereafter.
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, once weekly.
3.4.3	Food consumption	Yes, once weekly.
3.4.4	Water consumption	No
3.4.5	Functional observational battery	During week 4, grip strength and locomotor activity were assessed approx. 3 h after dosing:
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes, all surviving animals after 4 weeks on study and at the end of the recovery period, where applicable Parameters: erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, reticulocyte fluorescence ratios, nucleated erythrocytes (normoblasts), heinz bodies, methaemoglobin, total leukocyte count, differential leukocyte count, red blood cell morphology, thromboplastin time (=prothrombin time), activated partial thromboplastin time
3.4.8	Clinical chemistry	Yes, all surviving animals after 4 weeks on study and at the end of the recovery period, where applicable Parameters: glucose, urea, creatinine, uric acid, bilirubin, total cholesterol, total triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, alkaline phosphatase, gamma-glutamyl transferase, calcium, phosphorus, sodium, potassium, chloride, albumin, protein, total globulin, albumin/globulin ratio

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

3.4.9	Urinalysis	Yes, all surviving animals after 4 weeks on study and at the end of the recovery period, where applicable Parameters: volume (18-hour), specific gravity, osmolality, colour, appearance, pH, protein, glucose, ketone, bilirubin, blood, nitrite, urobilinogen, urine sediment, red blood cells, crystals (triple phosphate), crystals (uric acid)
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: brain, heart, liver, spleen, thymus, kidneys, adrenals, epididymides, testes
3.5.2	Gross and histopathology	Gross pathology: all dose groups; The following organs and tissues were collected from all animals at necropsy, organs in bold were examined histopathologically (control and high-dose only): **adrenal glands**, aorta, bone (sternum, femur including joint), **bone marrow** (femur), **brain** (cerebrum, cerebellum, pons), **caecum, colon, duodenum, epididymides** (fixed in Bouin's solution), oesophagus, eyes with optic nerve (fixed in Davidson's solution), Harderian gland (fixed in Davidson's solution), **heart, ileum with Peyer's patches, jejunum with Peyer's patches, kidneys**, larynx, lachrymal gland (exorbital), **liver, lungs (infused with formalin at necropsy), lymph nodes (mesenteric, mandibular)**, mammary gland area, nasal cavity, **ovaries**, pancreas, pituitary gland, **prostate gland, rectum**, salivary glands (mandibular, sublingual), **sciatic nerve, seminal vesicles**, skeletal muscle, skin, s**pinal cord (cervical, midthoracic, lumbar), spleen, stomach, testes (fixed in Bouin's solution), thymus, thyroid (w/ parathyroid gland)**, tongue, trachea, **urinary bladder (infused with formalin at necropsy), uterus, vagina, gross lesions**
3.5.3	Other examinations	–
3.5.4	Statistics	The following statistical methods were used to analyze grip strength, locomotor activity, body weights, organ weights and all ratios, as well as clinical laboratory data: – The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex. – The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution. – Fisher's exact-test was applied to macroscopical findings.
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	Clinical signs	Slight salivation was noted in two males and one female treated with 150 mg/kg and in several males and females treated with 750 mg/kg. This finding was considered to be test article-related. All other findings (piloerection, hunched posture, sedation, hypothermia, dyspnoea, breathing noise, vocalisation, kinked tail, hardened abdomen) were observed infrequently in individual animals and were considered to be common findings unrelated to the treatment with the test article.

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

		From treatment day 9 onwards, soft faeces (slight in degree) were noted in all animals. This finding was considered to be a common effect caused by the vehicle, PEG 300.
4.1.2	Mortality	One male (50 mg/kg) was found dead on treatment day 12. This animal showed only soft faeces for three days before being found dead. At necropsy, the lungs of this animal were incompletely collapsed. This finding was considered to be indicative of a dosing error. One female (750 mg/kg) was found dead on treatment day 9. Clinical signs observed on treatment day 7 included piloerection and hypothermia, whereas piloerection, hunched posture, sedation, dyspnoea and hypothermia were noted on day 8. At necropsy, none of the macroscopical findings (distension of the intestine and reduced spleen size) indicated a possible cause of death. The death of this animal was considered to be incidental. All other animals survived until scheduled necropsy.
4.1.3	Functional observational battery	Grip strength: No test article-related differences to the control values were ascertained. The hindlimb grip strength of the males treated with the test article at 750 mg/kg was significantly greater ($p<0.05$) than that of the controls. As no differences were noted in the females treated with 750 mg/kg, this finding was considered to be incidental. Locomotor activity: Reduced locomotor activity was noted in the males and females treated at 750 mg/kg and was considered to be related to the treatment with the test article. During all measurement intervals, the males and females treated with the test article at 750 mg/kg were less active than those of the control animals. The differences to the control values attained statistical significance in females (0-15 minutes, $p<0.05$; total locomotor activity, $p<0.05$) and males (15-30 minutes, $p<0.01$; 45-60 minutes, $p<0.05$; total locomotor activity, $p<0.01$). The locomotor activity of the animals treated at 50 or 150 mg/kg compared favourably with the control values.
4.2	**Body weight gain**	At 750 mg/kg, the mean body weight of males on treatment day 8 was significantly less ($p<0.01$) than that of the control males. The body weight gain from days 1 was +15.5%, compared with +20.9% of the control males. This finding correlated with the lower food consumption noted during the first treatment week and was considered to be test article-related. The mean body weights of the females treated with 750 mg/kg were lower than those of the control females throughout the treatment period, although the body weight gain compared favourably. Therefore, the difference in body weights was considered to be fortuitous. The body weight development of animals treated with 50 mg/kg or 150 mg/kg were unaffected when compared with the control values.
4.3	**Food consumption and compound intake**	At 750 mg/kg, food consumption was reduced in the males during the first treatment week and in the females throughout the study (treatment weeks 1-4). The mean daily food consumption of males and females treated with 50 mg/kg or 150 mg/kg were not affected by treatment. During the recovery period, the mean daily food consumption of all groups compared favourably.
4.4	**Ophthalmoscopic**	Not performed.

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

	examination	
4.5	**Blood analysis**	
4.5.1	Haematology	In males treated with 750 mg/kg the activated partial thromboplastin time was prolonged. This difference attained statistical significance and was considered to be test article-related. After the two-week recovery period, this parameter had returned to control levels. Although the activated partial thromboplastin times of the males treated with 50 mg/kg or 150 mg/kg were also longer than those of the control males, these values compared favourably with the historical data and the differences were therefore considered to be incidental. The few remaining differences between test article-treated and control animals were considered to be fortuitous.
4.5.2	Clinical chemistry	Test article-related differences noted in the clinical biochemistry parameters included increased plasma uric acid concentration in males ($p<0.01$) and females ($p<0.01$) at 750 mg/kg; reduced concentration of total plasma bilirubin in males ($p<0.01$) and females (not significant) treated with 750 mg/kg and in males ($p<0.01$) treated with 150 mg/kg; increased triglycerides in males ($p<0.01$) and females ($p<0.01$) treated with 750 mg/kg, increased phospholipids in females ($p<0.01$) treated with 750 mg/kg; increased alkaline phosphatase activity in males ($p<0.01$) treated at 750 mg/kg; reduced plasma chloride level in males (not significant) and females ($p<0.01$) treated at 750 mg/kg. After the two-week recovery period, the levels of phospholipids remained higher in males ($p<0.05$) and females (not significant) treated with 750 mg/kg, whereas total bilirubin remained lower in males ($p<0.05$) and females (not significant). The few remaining differences between test article-treated and control animals were either not dose-dependent or restricted to males or females and therefore considered to be fortuitous.
4.6	**Urinalysis**	Increased urine volume (18-hour), and decreased specific gravity and osmolality were noted in animals treated with the test article at 750 mg/kg. The differences attained statistical significance (males: $p<0.05$; females: $p<0.01$) and were considered to be test article-related. All other parameters were considered to be unaffected by treatment with the test article.
4.7	**Sacrifice and pathology**	
4.7.1	Organ weights	<u>After 4 Weeks:</u> Test article-related changes were restricted to increased absolute liver weights in the males and females treated with 750 mg/kg. The relative liver weights were also significantly increased (males: liver/body weight ratio, $p<0.01$ and liver/brain weight ratio, $p<0.05$; females: liver/body weight ratio, $p<0.05$) when compared with those of the control group. All other differences to the control values were considered to be incidental. Relative epididymides weights were also significantly increased in the mid and high dose group after 4 weeks. Absolut epididymides and epdidymides / brain ratio were significantly increased in the mid dose only. <u>After 6 Weeks:</u> After the 14-day recovery period, absolute liver weights remained higher in males treated with 750 mg/kg. Absolute and relative adrenal weights were reduced with statistical significance in males treated with 750 mg/kg. The absolute and relative organ weights of all females compared favourably.

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

See pp. 8 9-92 and 239-250

4.7.2 Gross necropsy and histopathology

Gross necropsy: All macroscopic findings recorded at both necropsies were considered to be spontaneous in nature and did not distinguish treated rats from the controls.

Histopathology: Test article-related findings were noted in the liver and stomach from rats treated with 750 mg/kg/day. In the liver, minimal or slight degrees of hepatocellular (midzonal/centrilobular) hypertrophy were recorded in four males and three females. In the stomach of rats treated with 750 mg/kg/day, hyperplasia/hyperkeratosis of the squamous epithelium of the forestomach was seen at minimal to moderate degrees in all animals of both sexes. None of these findings were seen following the recovery period.

Definitive causes of death could not be determined for the two unscheduled deaths. They were not considered to be test article-related.

The remainder of microscopic findings recorded at both necropsies were within the range of background pathology encountered in Wistar rats of this age and strain and occurred at similar incidences in control and test article-treated rats.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this subacute toxicity study, DCPP was administered daily by oral gavage to Wistar rats of both sexes at dose levels of 0, 50, 150 and 750 mg/kg body weight/day for a period of 28 days.

The groups comprised 5 animals per sex which were sacrificed after 28 days of treatment. Additional 5 rats per sex and group were used at 0 and 750 mg/kg. These animals were treated for 28 days and then allowed a 14-day treatment-free recovery period after which they were sacrificed.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during pre-test, the treatment and recovery periods. Functional observational battery, locomotor activity and grip strength were performed during week 4.

At the end of the dosing and the treatment-free recovery period, blood samples were withdrawn for haematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on selected organs and tissues from all control and high dose animals, as well as all gross lesions from all animals. From the animals of the low- and mid-dose groups, the livers and stomachs were examined to establish a no-effect level.

5.2 Results and discussion

Two animals died during the treatment period. Neither death was considered to be related to systemic toxicity caused by the test article.

All other animals survived until scheduled necropsy.

Slight salivation was noted with increasing incidence in animals treated with 150 mg/kg and 750 mg/kg. This finding was considered to be test article-related.

All other findings were considered to be related to the vehicle (i.e. soft faeces) or common findings unrelated to the treatment with the test article.

No test article-related differences in grip strength to the control values were noted.

A test article-related reduction of locomotor activity was noted in animals

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

		treated with the test article at 750 mg/kg.	
		A transient (during week 1 only) reduction of mean daily food consumption was noted in males treated with the test article at 750 mg/kg, whereas females treated at this dose level had reduced food consumption throughout the study. This finding was considered to be test article-related.	
		During treatment week 1, the body weight development of males treated at 750 mg/kg was generally lower than that of the controls. This finding was considered to be test article-related.	
		During subsequent weeks, the body weight development was generally similar to that of the controls. The body weight gain of all other animals was considered to be unaffected when compared with the controls.	
		The activated partial thromboplastin time was prolonged in males treated with the test article at 750 mg/kg, when compared with the controls. This finding was considered to be test article-related.	
		Test article-related differences noted in the clinical biochemistry parameters were restricted to increased plasma uric acid concentration (750 mg/kg); reduced concentration of total plasma bilirubin (males at 150 mg/kg and both sexes at 750 mg/kg), increased triglycerides (750 mg/kg); increased phospholipids (females at 750 mg/kg); increased alkaline phosphatase activity (males at 750 mg/kg); reduced plasma chloride level (750 mg/kg).	
		After the two-week recovery period, higher levels of phospholipids and lower levels of total bilirubin persisted in males ($p<0.05$) and females (not significant) treated with 750 mg/kg.	
		The few remaining differences to control values were considered to be incidental.	
		Test article-related differences in urinalysis parameters (increased urine volume, decreased specific gravity and osmolality) to the control values were noted only in animals treated at 750 mg/kg.	
		Test article-related organ weight changes were restricted to increased absolute and relative liver weights in the males and females treated with 750 mg/kg.	
		No test article-related macroscopic findings were noted in any animal at necropsy.	
		Morphologic alterations due to the test article were present in liver and stomach of some animals after 28 days' treatment with 750 mg/kg/day. Minor degrees of hepatocellular hypertrophy were seen in the liver, and hyperplasia / hyperkeratosis of the squamous epithelium of the forestomach. These findings were reversible during the recovery phase. No test article-related morphologic changes were noted in animals treated with 50 mg/kg/day or 150 mg/kg/day.	x
5.3	**Conclusion**		
5.3.1	LO(A)EL	750 mg/kg bw/day, based on clinical signs, increased liver weight/ hepatocellular hypertrophy and effects on urinalysis parameters	x
5.3.2	NO(A)EL	150 mg/kg bw/day	
5.3.3	Reliability	1	
5.3.4	Deficiencies	None	

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2011
Materials and Methods	Agree with the applicant's version.
Results and discussion	5.2: *Addition* In some cases the alteration of the forestomach was accompanied by minor degrees of inflammation. The effects on the forestomach might represent the response to the irritant effect (local) of the test substance.
Conclusion	5.3: LO(A)EL: *Addition:* based on reduced locomotor activity, hematology and clinical biochemistry Agree with the applicant's version with the amendments above.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

Table A6_3_1-1. Results of clinical chemistry, haematology and urinalysis (after 4 weeks)

Parameter	Control		50 mg/kg/day		150 mg/kg/day		750 mg/kg/day		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	5	5	5	5	5	5	5	5		
Haematology										
Thromboplastin time [s]	18.9	19.0	19.5	20.9	24.5	18.8	32.6**	19.9	+	–
Clinical chemistry										
Plasma uric acid [µM/L]	14.9	22.8	16.2	26.1	15.2	29.7	25.1**	43.5**	+	+
Total bilirubin [µM/L]	1.55	1.37	1.26	1.32	0.89**	1.38	0.70**	0.93	+	–
Triglycerides [mM/L]	0.25	0.23	0.45*	0.28	0.38	0.36	0.52**	0.47**	+	+
Alk. Phosphatase [µkat/L]	4.87	2.83	4.95	2.91	5.00	2.00	9.23**	2.93	+	–
G. glutamyl transferse [µkat/L] #	13.29	9.45	12.54	12.19	10.84	8.03	6.63**	6.8		
Chloride [mM/L]	100.9	102.0	100.9	103.8	100.7	102.7	97.9	97.8**	–	+
Potassium [mM/L]	3.21	2.94	3.08	2.99	3.22	2.99	2.65**	2.86	+	–
Urinalysis										
18-h Volume [~~mM~~mL]	5.6	3.5	5.3	4.4	6.3	3.4	9.0*	8.7**	+	+
spec. gravity [1]	1.068	1.063	1.073	1.052	1.057	1.071	1.050*	1.035*	+	+
Osmolality [mmol/kg]	1845	2042	1987	1687	1578	2140	1346*	1050**	+	+

*p< 0.05, **p<0.01

However, values within the historical range of data of the same rat strain

Section 6.3.1 Repeated dose toxicity

Annex Point IIA VI.6.3.1 6.3.1 Four-week oral toxicity study in rats

Table A6_3_1-2. Results of repeated dose toxicity study

Parameter	Control		50 mg/kg/day		150 mg/kg/day		750 mg/kg/day		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	5	5	5	5	5	5	5	5		
Body weight, terminal [g]	283.5	168.7	288.3	170.0	277.1	170.5	261.6*	161.8	+	–
Organ weights										
Liver, % bw	2.92	3.18	3.06	3.56	3.22	3.27	3.50**	3.76*	+	+
FOB										
Locomotor activity, low beam counts	1991	2262	1779	2695	1797	2629	1028**	1378*	+	+
Histopathology										
Liver, hepato-cellular hypertrophy	0/5[a]	0/5	0/5	0/5	0/5	0/5	4/5	3/5	+	+
Forestomach, squamous hyper-plasia	0/5	0/5	0/5	0/5	0/5	0/5	5/5	5/5	+	+
Forestomach, inflammation	0/5	0/5	0/5	0/5	0/5	0/5	2/5	2/5	+	+

*p< 0.05, **p<0.01

[a] number of animals affected/total number of animals

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (2001): [redacted]B: 28 Day Dermal Toxicity Study in Rats (OECD EU). [redacted] Report No. [redacted] date: 2001-06-13 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, EC Method B.9 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	The highest dose level did not produce any sign of toxicity.
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Beige, off white powder
3.1.2.2	Purity	>99%(w/w)
3.1.2.3	Stability	Expiry date: August 2009 Stability in vehicle was verified by analysis.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Alpk:AP_fSD (Wistar-derived)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ and ♀
3.2.5	Age/weight at study initiation	Approximately 5 weeks old on delivery Weight: 201-227 g (♂), 197-230 g (♀)
3.2.6	Number of animals per group	5/sex/dose level
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	28 days
3.3.2	Frequency of exposure	Daily (5 days/week = 20 applications)
3.3.3	Post-exposure period	14 days, control and high-dose satellite group (5/sex/group)

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

3.3.4	**Dermal**	
3.3.4.1	Area covered	About 10% of body surface
3.3.4.2	Occlusion	Occlusive
3.3.4.3	Vehicle	Propylene glycol
3.3.4.4	Concentration in vehicle	1.5, 5.0, 15 mg/mL
3.3.4.5	Total volume applied	2 mL/kg bw
3.3.4.6	Dose applied	3, 10, 30 mg/kg bw
3.3.4.7	Duration of exposure	6 h/day
3.3.4.8	Removal of test substance	Yes, wiping with water and cotton swabs
3.3.4.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily
3.4.1.2	Mortality	Yes, twice daily (on weekends and holidays: once daily).
3.4.2	Body weight	Yes, once daily
3.4.3	Food consumption	Yes, continuously
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: red blood cell count, haemoglobin, total white cell count, haematocrit, platelet count, mean cell volume, mean cell haemoglobin concentration, mean cell haemoglobin, differential white cell count, blood cell morphology including differential white cell count, prothrombin time and activated partial thromboplastin time.
3.4.7	Clinical chemistry	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: glucose, creatine kinase activity, urea, gamma-glutamyl transferase activity, creatinine, sodium, albumin, potassium, total protein, chloride, albumin/globulin ratio, phosphorus (as phosphate), total bilirubin, calcium, alkaline phosphatase activity, cholesterol, alanine aminotransferase activity, triglycerides, aspartate aminotransferase activity
3.4.8	Urinalysis	Yes, all animals prior to main study termination and all satellite animals prior to satellite termination. Parameters: colour , glucose, volume, ketones, appearance, bilirubin, specific gravity, protein, pH, blood

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes, from all animals sacrificed at termination. Organs: adrenal glands, liver, kidneys, testes
3.5.2	Gross and histopathology	All animals were sacrificed at study termination and a gross pathological examination was performed. Histopathology: from all animals of the control and highest dose group (main study) Organs: abnormal tissue, skin (treated), spleen, adrenal gland, skin (untreated), heart, kidney, testis, liver, epididymis
3.5.3	Other examinations	–
3.5.4	Statistics	Bodyweights were considered by analysis of covariance on initial (day 1) bodyweight, separately for males and females. Weekly food consumption was considered by analysis of variance, separately for males and females. Haematology and blood clinical chemistry and urine clinical chemistry were considered by analysis of variance. Male and female data were analysed together and the results examined to determine whether any differences between control and treated groups were consistent between the sexes. Organ weights were considered by analysis of variance and analysis of covariance on final bodyweight, separately for males and females. Summary data are presented for organ to bodyweight ratios but these were not analysed statistically as the analysis of covariance provides a better method of allowing for differences in terminal bodyweights. All analyses were carried out separately for the main study and recovery groups. Analyses of variance and covariance for main study groups allowed for the replicate structure of the study design and were carried out using the MIXED procedure in SAS (1996). Least-squares means for each group were calculated using the LSMEAN option in SAS PROC MIXED. Unbiased estimates of differences from control were provided by the difference between each treatment group least-squares mean and the control group least-squares mean. Differences from control were tested statistically by comparing each treatment group least-squares mean with the control group least-squares mean using a two-sided Student's t-test, based on the error mean square in the analysis.
3.6	**Further remarks**	–

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	Treatment-related clinical changes, e.g. oedema and skin sensitive to touch, were seen sporadically in most treated male groups and in 1 top dose female towards the end of the dosing period. These changes did not persist into the recovery phase and are considered to be of no toxicological significance. A number of clinical observations, e.g. thickening, desquamation, chromodacryorrhea, erythema, small scattered scabs and scabs at the edge of the application area, consistent with those commonly seen in dermal toxicity studies as a consequence of bandaging, were seen in

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

		control animals at a similar incidence to the treated groups and/or showed no evidence of a dose-response and are considered to be unrelated to administration of DCPP.
4.1.2	Mortality	There were two mortalities; one male given 30 mg/kg/day and one female given 3 mg/kg/day were found dead on days 4 and 5 respectively. In the absence of any clinical change, effects on bodyweight or evidence of a dose-response these deaths are considered to be unrelated to treatment with DCPP.
4.2	**Body weight gain**	Group mean bodyweights in high-dose males given were slightly lower than the control values but the difference seldom achieved statistical significance (main study 7% and recovery group 12% lower than the concurrent control values, see Tables A6_3_2-1 and -2). No effect on body weight was noted during the recovery period.
4.3	**Food consumption and compound intake**	Food consumption in all groups was generally similar to control values. However individual males in the 30 mg/kg/day main and recovery showed some reduction in food consumption.
4.4	**Blood analysis**	
4.4.1	Haematology	There were statistically significant differences from control values in some haematological parameters. These were slight, were present in the recovery group (30 mg/kg) only and /or showed no evidence of a dose-response relationship and therefore are considered to be unrelated to treatment with DCPP.
4.4.2	Clinical chemistry	There were statistically significant differences from control values in some blood clinical chemistry parameters. These were slight, were present in the recovery group (30 mg/kg) only and/or showed no evidence of a dose-response relationship and therefore are considered to be unrelated to treatment with DCPP.
4.4.3	Urinalysis	There were statistically significant differences from control values in some urine clinical chemistry parameters. These were slight and were present in the recovery group only, and therefore are considered to be unrelated to treatment with DCPP.
4.5	**Sacrifice and pathology**	
4.5.1	Organ weights	No dose-related effects.
4.5.2	Gross and histopathology	Gross pathology: no treatment-related effect. Histopathology: no treatment-related effect.
4.6	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of five male and five female Alpk:AP$_f$SD (Wistar-derived) rats were administered 3, 10 or 30mg DCPP/kg bw/day (in propylene glycol) by dermal application over a 28 day period (20 applications). A concurrent control group was similarly treated, but propylene glycol only was applied. Two additional groups were similarly dosed with propylene glycol or 30 mg DCPP/kg bw/day and then retained without treatment for a further 14 days (recovery phase). Clinical observations, bodyweights and food consumption were recorded throughout the study. Urine samples were taken during weeks 4 (main study) and 6 (recovery phase) for clinical pathology. At the end

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

		of the scheduled period, the animals were killed and subjected to an examination *post mortem*. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathology examination.
5.2	**Results and discussion**	At a dose level of 30 mg DCPP/kg bw/day bodyweight and bodyweight gain, in males only, was slightly lower than controls throughout the dosing period; there was evidence of recovery. There were no other compound-related effects in males at this dose level or in either sex at any dose level. All groups, including controls, showed some clinical changes generally associated with dermal administration of a test material.
5.3	**Conclusion**	Dermal administration of 30 mg DCPP/kg bw/day for 28 consecutive days (20 applications) produced effects (reduced bodyweight gain) in male rats only. There was evidence of recovery on cessation of treatment. There was no evidence of skin irritancy at this dose level. The NOEL for DCPP was 10 mg/kg/day. Since the observed reduction in body weight gain was hardly statistically significant and without any functional or morphological correlate, it is not considered an adverse effect.
5.3.1	LO(A)EL	LOAEL > 30 mg/kg bw/day, no treatment-related adverse effects
5.3.2	NO(A)EL	NOAEL = 30 mg/kg bw/day
5.3.3	Other	–
5.3.4	Reliability	2
5.3.5	Deficiencies	The highest dose level was insufficient to produce toxicity. However, it is unlikely that specific signs of systemic toxicity would have been produced at a higher dose as judged from the low toxicity in both the subacute oral and the acute dermal toxicity test with DCPP.

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2013-07-26
Materials and Methods	Agree with applicant´s version.
Results and discussion	Agree with applicant´s version.
Conclusion	Agree with applicant´s version.
Reliability	2
Acceptability	acceptable
Remarks	

Section 6.3.2 Repeated dose toxicity

Annex Point IIA VI.6.3.2 6.3.2 28-day dermal toxicity study in rats

Table A6_3_2-1. Calculation of body weight gains [g]

	Males					
	Main				Recovery	
	0	3	10	30	0	30
d43					417.1	372.6
d28	333.1	325.6	330.7	323.2	342.3	294.8
d1	214.8	214.2	213.4	218.2	217.0	213.6
wk 1-5	118.3	111.4	117.3	105.0	125.3	81.2
wk 5-7					74.8	77.8
wk 1-7					200.1	159.0

	Females					
	Main				Recovery	
	0	3	10	30	0	30
d43					281.1	281.5
d28	260.2	268.9	256.2	265.2	259.2	263.4
d1	216.8	214.8	213.0	217.8	214.8	217.4
wk 1-5	43.4	54.1	43.2	47.4	44.4	46.0
wk 5-7					21.9	18.1
wk 1-7					66.3	64.1

Table A6_3_2-2. Results of repeated dose toxicity study

Parameter	Control		3 mg/kg/day		10 mg/kg/day		30 mg/kg/day		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	5	5	5	5	5	5	5	5		
bodyweight gain [g][1]										
weeks 1-5	118.3	43.4	111.4	54.1	117.3	43.2	105.0	47.4	+	–
weeks 1-7	200.1	66.3	/	/	/	/	159.0	64.1	+	–
weeks 5-7	74.8	21.9	/	/	/	/	77.8	18.1	–	–

[1] Differences between group means did not reach statistical significance

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2001), [redacted] 13-Week Oral Toxicity (Gavage) Study in Wistar Rats. [redacted] Project No. 7[redacted]3, date: 2001-04-18 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 408 (1998, = EC Method B.26 (1996))	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] = DCPP	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Description	Solid powder	
3.1.2.2	Purity	> 99%	
3.1.2.3	Stability	Expiration date: October 31, 2008 Stable under storage conditions Stability in vehicle was confirmed.	
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	HanIbm:WIST (SPF)	
3.2.3	Source	[redacted]	
3.2.4	Sex	♂ + ♀	
3.2.5	Age/weight at study initiation	Age: 4 weeks (at delivery) Weight range: 65-92 g (♂) and 53-67 g (♀).	
3.2.6	Number of animals per group	Control and high-dose group: 15 per sex Low- and mid-dose group: 10 per sex	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	91/92 days	
3.3.2	Frequency of exposure	Daily	
3.3.3	Post-exposure period	28 days (control and high dose only)	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

3.3.4	**Oral**		
3.3.4.1	Type	Gavage	
3.3.4.2	Doses	20, 100, 500 mg/kg bw/day	
3.3.4.3	Vehicle	PEG 300	
3.3.4.4	Concentration in vehicle	4, 20, 100 mg/mL	
3.3.4.5	Dose volume	5 mL/kg bw	
3.3.4.6	Controls	Vehicle	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, once daily (general cageside observations) or once weekly (detailed clinical observations)	
3.4.1.2	Mortality	Yes, twice daily	
3.4.2	Functional observations	Grip strength and locomotor activity were assessed in the last treatment week	
3.4.3	Body weight	Yes, once weekly.	
3.4.4	Food consumption	Yes, once weekly.	
3.4.5	Water consumption	Yes, once weekly.	
3.4.6	Ophthalmoscopic examination	Yes, at pre-test , at 13 and 17 weeks	x
3.4.7	Haematology	Yes, after 13 and 17 weeks Parameters: erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelet count, reticulocyte count, reticulocyte fluorescence ratios, nucleated erythrocytes (normoblasts), heinz bodies, methaemoglobin, total leukocyte count, differential leukocyte count, red blood cell morphology, thromboplastin time (=prothrombin time), activated partial, thromboplastin time	
3.4.8	Clinical chemistry	Yes, after 13 and 17 weeks Parameters: glucose, urea, creatinine, uric acid, bilirubin, total cholesterol, total triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, creatine kinase, alkaline phosphatase, gamma-glutamyl transferase, calcium, phosphorus, sodium, potassium, chloride, albumin, protein (total + electrophoresis), total globulin, albumin/globulin ratio	
3.4.9	Urinalysis	Yes, after 13 and 17 weeks Parameters: volume (18-hour), specific gravity, osmolality, colour, appearance, pH, protein, glucose, ketone, bilirubin, blood, nitrite, urobilinogen, urine sediment, white blood cells, crystals (triple phosphate), crystals (uric acid)	
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes Organs: brain, heart, liver, thyroids w/ parathyroids, spleen, thymus, kidneys, adrenals, uterus, epidydimides, testes, epididymides, ovaries	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

3.5.2	Gross and histopathology	Gross pathology: all dose groups The following organs and tissues were collected from all animals at necropsy, organs in bold were examined histopathologically (control and high-dose only): **adrenal glands**, **aorta**, bone (sternum, femur including joint), **bone marrow** (femur), **brain** (cerebrum, cerebellum, pons), **caecum, colon, duodenum, epididymides** (fixed in Bouin's solution), **oesophagus**, **eyes with optic nerve** (fixed in Davidson's solution), Harderian gland (fixed in Davidson's solution), **heart, ileum with Peyer's patches, jejunum with Peyer's patches, kidneys**, larynx, lachrymal gland (exorbital), **liver, lungs** (infused with formalin at necropsy)**, lymph nodes** (mesenteric, mandibular), **mammary gland area**, nasal cavity (turbinates), **ovaries**, **pancreas, pituitary gland, prostate gland, rectum**, **salivary glands** (mandibular, sublingual), **sciatic nerve, seminal vesicles**, skeletal muscle, **skin**, **spinal cord** (cervical, midthoracic, lumbar)**, spleen, stomach, testes** (fixed in Bouin's solution)**, thymus, thyroid (w/ parathyroid gland)**, tongue, **trachea, urinary bladder (infused with formalin at necropsy), uterus, vagina, gross lesions**	
3.5.3	Other examinations	–	
3.5.4	Statistics	The following statistical methods were used to analyze grip strength, locomotor activity, body weights, organ weights and all ratios, as well as clinical laboratory data and ophthalmoscopy: – The Dunnett-test (many to one t-test) based on a pooled variance estimate was applied if the variables could be assumed to follow a normal distribution for the comparison of the treated groups and the control groups for each sex. – The Steel-test (many-one rank test) was applied instead of the Dunnett-test when the data could not be assumed to follow a normal distribution. – Student's T-Test was applied to loco activity and grip strength. – Fisher's exact-test was applied to ophthalmoscopy data, and macroscopic findings. – Armitage/Cochran Trend Test for non-neoplastic lesions, if appropriate.	

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1	Clinical signs	Shortly after administration of the test article on treatment day 81, one female (no. 94) treated with 500 mg/kg/day was prostrate, had clonic spasms and noisy breathing. The animal recovered within one hour. Repeated incidences of breathing noises were noted in both sexes treated with 500 mg/kg/day, although more frequently in males. This finding was considered to be test article-related. No abnormal findings were noted during daily observations in males and females treated with 20 mg/kg/day. Incidences of breathing noises were noted in males treated with 100 mg/kg/day. Salivation was also noted intermittently in one female (no. 79) treated with 100 mg/kg/day and both sexes treated with 500 mg/kg/day. Piloerection was noted in single animals of both sexes treated with 500 mg/kg/day, whereas sedation was restricted to a single female (no. 94) treated with 500 mg/kg/day. These findings were generally slight in	x

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

		degree, transient and considered to be unrelated to the test article treatment.
4.1.2	Mortality	All animals survived until scheduled necropsy.
4.1.3	Functional observations	Grip Strength: The mean forelimb grip strength of the males treated with 500 mg/kg/day were significantly ($p<0.05$) lower than those of the control males. This finding was considered to be incidental as the hindlimb grip strength of these animals compared favorably with those of the control males. The fore- and hindlimb grip strength of the test article-treated females were similar to those of the controls females. Locomotor Activity: The 45-60 minute measurement interval of the males treated with 20 mg/kg/day or 100 mg/kg/day, as well as the 30-45 minute measurement interval of the females treated with 100 mg/kg/day were significantly less ($p<0.05$) than those of the controls. In the absence of a dose-response relationship, these changes were considered to be incidental. The total mean locomotor, activity of the remaining test article-treated animals compared favourably with those of the controls.
4.2	**Body weight gain**	The mean body weights of males treated with 100 mg/kg/day or 500 mg/kg/day were marginally lower than those of the control males during the treatment period. During week 9 of treatment only, the difference to control values attained statistical significance ($p<0.05$) at 500 mg/kg/day. Slightly lower body weight gain was noted during the treatment period in males treated with 500 mg/kg/day. The mean body weights of test article-treated females compared favourably with or exceeded those of the control females throughout the treatment period. The differences from the control values noted in the females treated with 500 mg/kg/day attained statistical significance during weeks 4-9 and 13. The mean body weight gain values of these females also exceeded that of the control females, attaining statistical significance during treatment weeks 4-7 In the absence of consistent findings in both sexes treated with 500 mg/kg/day, the differences from the control values during the treatment period were considered unlikely to be a test article-related effect. The females treated with 20 mg/kg/day had higher body weight gain during treatment weeks 2 and 4-7. In the absence of a dose-response relationship, these differences from the control values were considered to be incidental. During the recovery period, the mean body weights and mean body weight gain of the males previously treated with 500 mg/kg/day remained lower than those of the control males. None of the values attained statistical significance. Females treated previously with the test article had generally higher body weights and body weight gain throughout the recovery period.
4.3	**Food consumption**	The mean food consumption of the males treated with 100 mg/kg/day or 500 mg/kg/day was -3.8% and -3.4% lower, respectively, than that of the control males during the treatment period. The mean daily food consumption of the test article-treated females compared favourably with that of the control females throughout the treatment period. In the absence of similar findings in both sexes treated with 500 mg/kg/day, these differences from the control values were considered to be incidental. During the recovery period, the mean daily food consumption of the males previously treated with 500 mg/kg/day compared favourably with that of the control males, whereas the previously treated females consumed 13.2%

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

		more feed than the control females. The toxicological significance of the latter difference is unclear, but a relationship with the test article is considered to be unlikely.
4.4	**Water consumption**	The mean daily water consumption of the rats treated with 500 mg/kg/day exceeded that of the controls throughout the treatment period. The findings were more strongly expressed in females. During the recovery period, the mean daily water consumption remained higher in males during weeks 14 and 15 (i.e. weeks 1 and 2 of recovery) and in females during week 14 only. These differences were considered to be test article-related. The mean water consumption of rats treated with 20 mg/kg/day or 100 mg/kg/day compared favourably with the controls during the treatment period.
4.5	**Ophthalmoscopic examination**	No effects.
4.6	**Blood analysis**	
4.6.1	Haematology	The mean red blood cell count of the males treated with 100 mg/kg/day or 500 mg/kg/day were significantly lower ($p<0.05$ and $p<0.01$, respectively) than that of the control males after 13 weeks' treatment. These differences remained within the 95% tolerance limits of the historical control data, and in the absence of commensurate findings in related parameters, they were considered to be of equivocal toxicological significance. The absolute and relative reticulocyte counts were significantly lower ($p<0.05$ and $p<0.01$, respectively) in males treated with 500 mg/kg/day for 13 weeks when compared with the controls. Although the differences in these parameters attained statistical significance, the differences remained within the 95% tolerance limits of the historical control data and similar findings were not evident in females at this dose level. The fluorescent reticulocyte ratio was 'left-shifted' in females treated with 500 mg/kg/day for 13 weeks when compared with the controls. The ratio of high fluorescent reticulocytes was significantly higher ($p<0.01$) and the ratio of low fluorescent reticulocytes was marginally lower when compared with the controls. The normoblast count of females treated with 500 mg/kg/day was marginally higher than that of the controls. All values remained within the 95% tolerance limits of the historical control data, and a relationship with the treatment of the test article was considered to be unlikely. The thromboplastin and activated partial thromboplastin times of males treated with 500 mg/kg/day were significantly prolonged ($p<0.01$) when compared with the controls. In females treated with 100 mg/kg/day or 500 mg/kg/day, the thromboplastin times were significantly abbreviated when compared with the controls. These conflicting findings were considered to be incidental. The remaining haematology parameters of all groups recorded after 13 weeks' treatment compared favourably. All values were similar to those of the controls after four weeks' recovery.
4.6.2	Clinical chemistry	Higher levels of creatinine ($p<0.05$, males only) and lower levels of chloride were noted in males and females treated with 500 mg/kg/day after 13 weeks' treatment when compared with the control animals. Higher levels of triglycerides ($p<0.01$), higher levels of phospholipids and increased activities of alkaline phosphatase ($p<0.01$) were evident in males and females of the highest dose group when compared with the control animals.

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

These findings were considered to be test article-related changes in the kidneys and liver.

With exception of higher creatinine levels which persisted in females treated previously with 500 mg/kg/day, all differences reverted to levels generally comparable with the control values after the four-week recovery period.

All remaining differences from the control values were not supported by commensurate findings in related parameters, were without a clear dose-response relationship or not seen in the other sex, and therefore were considered likely to be fortuitous.

4.7 Urinalysis

Urine production was significantly increased in males treated with 100 mg/kg/day ($p<0.05$) and in both sexes treated with 500 mg/kg/day ($p<0.01$) when compared with the controls after 13 weeks. The urine of these animals also had lower specific gravity and osmolality ($p<0.05$ or $p<0.01$) when compared with the controls after 13 weeks. Amorphous urates were present and increased in the urine of males treated with 100 mg/kg/day or 500 mg/kg/day.

These changes were considered to be test article-related changes associated with alterations in the kidneys' ability to concentrate urine.

The differences from the control values noted in the urine pH of females treated with 500 mg/kg/day were statistically significant ($p<0.01$), but were not supported by a dose response relationship and therefore considered to be incidental.

After the four-week recovery period, all differences reverted to levels which were generally similar to the control group.

4.8 Sacrifice and pathology

4.8.1 Organ weights

<u>After 13 weeks:</u> The mean absolute liver weights and ratios were higher in males and females treated with 500 mg/kg/day. The differences from the control values attained statistical significance and were considered to be test article-related.

A trend for slightly higher absolute and relative kidney weights was noted in females treated with 500 mg/kg/day. Marginally higher kidney-to-body weights ($p<0.05$) were noted in males treated with 500 mg/kg/day. A relationship with the test article treatment was considered equivocal.

The slightly lower brain weight noted in the males treated with 500 mg/kg/day was not seen in the females of this dose group and therefore considered to be unrelated to the test article.

All remaining differences from the control values were considered to be incidental.

<u>After 17 weeks:</u> no differences between high-dose and control recovery group.

4.8.2 Gross and histopathology

<u>Macroscopic Findings</u>

In one female treated with 100 mg/kg/day and three females treated with 500 mg/kg/day, tan coloration of the adrenals was noted after 13 weeks.

The remaining findings were considered to be within the range of spontaneous background alterations in rats of this strain and age. They consisted of incompletely or non-collapsed lungs, foamy fluid released from the bronchi, hardened papillary process of the liver, renal pelvis dilation, testes reduced in size, thickened thyroid glands, size reduction or thickening of the thymus, uterus horn dilation, ovaries reduced in size or

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

water cysts on ovaries, discoloration and/or discoloured foci in several organs.

Microscopic Findings

Oesophagus: Epithelial hyperplasia (slight in degree) was recorded in two males treated with 500 mg/kg/day for 13 weeks compared with two control males. In addition, there was minimal to slight hyperkeratosis noted in two control males, one male and one female treated with 20 mg/kg/day, three males and one female treated with 100 mg/kg/day and all males and seven females treated with 500 mg/kg/day for 13 weeks. This finding was statistically significant ($p<0.0005$) in the trend test for non-neoplastic lesions (Armitage).

Stomach: After 13 weeks, a slightly higher incidence of hyaline droplets was observed in the glandular mucosa (adjacent to the limiting ridge) of animals treated with 100 mg/kg/day or 500 mg/kg/day. Such incidences were not recorded in animals of the recovery group.

Furthermore, lymphoid follicles in the submucosa and increased inflammatory cell infiltrate were recorded at higher incidences in males treated with 100 mg/kg/day, and in animals treated with 500 mg/kg/day after 13 weeks' treatment ($p=0.0446$ in males, Trend test for non-neoplastic lesions according to Armitage) and after 4 weeks' recovery. These findings were accompanied by minimal to moderate forestomach hyperkeratosis in eight males and three females treated with 100 mg/kg/day and all animals treated with 500 mg/kg/day for 13 weeks ($p<0.0005$, Trend test for non-neoplastic lesions according to Armitage). Hyperkeratosis (minimal to slight in degree) was recorded in two males ($p=0.0071$) and four females previously treated with 500 mg/kg/day.

Epithelial hyperplasia was recorded in two males treated with 100 mg/kg/day and in all animals treated with 500 mg/kg/day for 13 weeks ($p<0.0005$). This finding persisted in the latter animals after 4 weeks' recovery ($p=0.0014$).

Liver: Hepatocellular hypertrophy (minimal to slight in degree) was noted in ten males ($p<0.0005$) and three females ($p=0.0301$) each in groups treated with 100 mg/kg/day or 500 mg/kg/day after 13 weeks (see Table A6_4_1-2).

Pancreas: Increased vacuolation with dense bodies (minimal to slight in degree) was noted in three males treated with 500 mg/kg/day after 13 weeks.

Kidneys: Hyaline droplets in tubular cells were recorded at slightly higher severity in males treated with 500 mg/kg/day for 13 weeks when compared with the controls. Seven males ($p<0.0005$) and three females ($p<0.0012$) treated with 500 mg/kg/day for 13 weeks showed multifocal tubular cell swelling (minimal to moderate in degree) accompanied by tubular cell necrosis (minimal degree) in three males ($p=0.0012$). All of the aforementioned findings did not persist in the animals of the recovery group. However, lymphoid cell foci increased in incidence in recovery males and attained statistical significance ($p=0.0071$).

Other Findings: There was no histological correlate to the tan discoloration of the adrenals.

All findings not described above were considered to be within the range of background lesions which may be recorded in rats of this strain and age.

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

In this subchronic toxicity study, DCPP was administered daily by oral gavage to SPF-bred Wistar rats of both sexes at dose levels of 20, 100 and 500 mg/kg body weight/day for a period of 90 days. A concurrent control group was treated similarly with the control article, PEG 300, only.

The groups comprised 10 animals per sex which were sacrificed after 90 days of treatment. Additional 5 rats per sex and group were used at 0 and 500 mg/kg. These animals were treated for 90 days and then allowed a 28-day treatment-free recovery period after which they were sacrificed.

Clinical signs, outside cage observation, food consumption and body weights were recorded periodically during pretest, the treatment and recovery periods. Ophthalmoscopic examinations were performed at pretest, the end of the treatment and recovery periods. Functional observational battery, locomotor activity and grip strength were performed during week 13.

At the end of the dosing and the treatment-free recovery period, blood samples were withdrawn for haematology and plasma chemistry analyses. Urine samples were collected for urinalyses. All animals were killed, necropsied and examined post mortem. Histological examinations were performed on organs and tissues from all control and high dose animals, and all gross lesions from all animals.

From the animals of the low- and middle-dose groups, pancreas (males only), oesophagus, stomach, kidneys and liver were examined to establish a no-effect level.

5.2 Results and discussion

All animals survived until scheduled necropsy.

Increased incidences of breathing noises were noted during daily observations and weekly observations (performed weeks 1-12) in both sexes treated with 500 mg/kg/day, although more frequently in males. This finding was considered to be test article-related, although no morphological correlate was evident.

All remaining clinical signs were considered to be unrelated to treatment.

Breathing noises were not evident in the animals allocated to the recovery group.

Breathing noises were noted during the functional observational battery (performed week 13) in both sexes treated with 500 mg/kg/day, although more frequently in males. This finding was considered to be test article-related. All remaining clinical signs were considered to be unrelated to test article.

No test article-related changes in the fore- or hindlimb grip strength were noted at any dose level.

No test article-related differences were noted in the mean locomotor activity (measured over 60 minutes) of the control and test article-treated animals.

Minor differences noted in the mean daily food consumption of test article-treated and control animals were considered unlikely to be related to the treatment with the test article.

A test article-related increase in water consumption was noted in males and females treated with 500 mg/kg/day. These changes often attained statistical significance: Although the differences were more clearly expressed in females during the treatment period, the mean water

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

consumption remained slightly elevated for the first two weeks of recovery in males and for the first week of recovery for females.

The mean water consumption values of rats treated with the test article at 20 mg/kg/day or 100 mg/kg/day were similar to those of the control rats during the treatment period.

Minor differences noted in the body weight development of test article-treated and control animals were considered unlikely to be related to the treatment with the test article.

No test article-related ophthalmologic findings were noted when compared with the control animals.

Findings noted in the haematology parameters of animals treated with 100 mg/kg/day or 500 mg/kg/day were not supported by commensurate changes in related parameters, or remained within the 95% tolerance limits of the historical control data, and were therefore considered to be without toxicological significance.

When compared with control values, creatinine levels were higher and chloride levels were lower in males and females treated with 500 mg/kg/day were considered to be indications of effects upon the kidneys, whereas higher levels of triglyceride, phospholipids and increased activity of alkaline phosphatase seen in these animals were related to hepatic effects.

During recovery, higher creatinine levels persisted in the females previously treated with 500 mg/kg/day. All remaining differences reverted to control levels.

Increased urine production, lower specific gravity and lower osmolality noted in males and females treated with 500 mg/kg/day when compared with controls, indicating test article- related changes considered to be associated with alterations in the kidneys' ability to concentrate urine. These changes were reversible after four weeks' recovery.

The mean absolute and relative liver weights of males and females treated with the test article for 13 weeks at 500 mg/kg/day were higher than those of the controls, and considered to be test article-related. This difference reverted to levels similar to that of the controls after four weeks' recovery.

All other organ weights and ratios were unaffected.

No test article-related macroscopic findings were noted at any dose level.

Morphologic differences from the control animals were noted in the oesophagi, stomachs, kidneys, livers of males and females treated with 500 mg/kg/day, whereas changes in the pancreas were limited to males treated with 500 mg/kg/day. Most of these findings were reversible after the recovery period.

The findings in the oesophagus consisted of epithelial hyperplasia in some animals treated with 500 mg/kg/day for 13 weeks. Hyperkeratosis of the oesophagus was increased in incidence in animals treated with 100 mg/kg/day or 500 mg/kg/day.

In the stomach of animals treated with 500 mg/kg/day for 13 weeks, the severity of hyaline droplets in the glandular stomach was slightly increased. Submucosal lymphoid follicles and inflammatory cell infiltrate was increased in incidence in these animals after treatment and after recovery. Forestomach hyperkeratosis and epithelial hyperplasia were noted in some animals treated with 100 mg/kg/day and all animals treated with 500 mg/kg/day. This finding persisted after recovery in some animals of the latter group.

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

		In the liver of animals treated for 13 weeks, hepatocellular hypertrophy was noted in ten males and three females treated with 100 mg/kg/day and ten males and three females treated with 500 mg/kg/day. This finding was reversible in the latter group after recovery. In the pancreas, increased vacuolation with dense bodies were recorded in three males treated with 500 mg/kg/day for 13 weeks. The kidneys of males treated with 500 mg/kg/day showed a slightly higher grade of severity for hyaline droplets after 13 weeks. Multifocal tubular swelling was seen in several males treated with 500 mg/kg/day after 13 weeks, occasionally accompanied by tubular cell necrosis. Although these kidney findings were reversible after 13 weeks, the incidence of lymphoid cell foci increased after recovery in males treated with 500 mg/kg/day.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	100 mg/kg bw/day, based on morphological changes in various tissues and on functional effects on kidney	x
5.3.2	NO(A)EL	20 mg/kg bw/day	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2010
Materials and Methods	3.4.6: *Addition:* low/high dose
Results and discussion	4.1.1: *Amendment:* Text-article related changes included breathing noise noted on four occasions in males treated with 100 mg/kg/day, on ten occasions in males treated with 500 mg/kg/day and on three occasions in females treated with 500 mg/kg/day.
Conclusion	5.3.1 Addition: tissues: esophagus, stomach, liver, kidney, clinical signs (breathing noise)
Reliability	1
Acceptability	Acceptable
Remarks	-

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

Table A6_4_1-1. Results of clinical chemistry, haematology and urinalysis (after 13 weeks)

Parameter	Control		20 mg/kg		100 mg/kg		500 mg/kg		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	10	10	10	10	10	10	10	10		
Haematology										
RBC count	–	–	–	–	↓	–	↓	–	+	–
Reticulocytes	–	–	–	–	–	–	↓	–	+	–
Thromboplastin time	–	–	–	–	–	↓	↑	↓	+	+
Partial thromboplastin time	–	–	–	–	–	–	↑	–	+	–
Clinical chemistry										
Phospholipids*	–	–	–	–	–	–	↑	↑	+	+
Triglycerides	–	–	–	–	–	–	↑	↑	+	+
AP	–	–	↑	–	–	–	↑	↑	+	+
Creatinine	–	–	–	–	–	–	↑	–	+	–
Chloride	–	–	–	–	–	–	↓	↓	+	–
Urinalysis										
Volume	–	–	–	–	↑	–	↑	↑	+	+
Spec. gravity	–	–	–	–	–	–	↓	↓	+	+
Osmolality	–	–	–	–	–	–	↓	↓	+	+

↑, ↓: statistically significant increase and decrease, respectively
– : no significant difference from control values
*inserted by the RMS

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (01) 13-week oral toxicity study in rats

Table A6_4_1-2. Results of repeated dose toxicity study (after 13 weeks)

Parameter	Control		20 mg/kg		100 mg/kg		500 mg/kg		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	10	10	10	10	10	10	10	10		
Water consumption	–	–	–	–	–	–	↑	↑	+	+
Organ weights										
Liver, abs.	–	–	–	–	–	–	↑	↑	+	+
Liver, rel.	–	–	–	–	–	–	↑	↑	+	+
Kidneys, rel.	–	–	–	–	–	–	↑	–	+	–
Gross necropsy										
Adrenals, tan discolouration	0[a]	0	0	0	0	1	0	3	–	+
Histopathology										
Oesophagus, epithelial hyperplasia	0	0	0	0	0	0	2	0	+	–
Oesophagus, hyperkeratosis	2	0	1	1	3	1	10	7	+	+
Forestomach, hyperkeratosis	0	0	0	0	8	3	10	10	+	+
Forestomach*, epithelial hyperplasia	0	0	0	0	2	0	10	10	+	+
Stomach, erosion	0	1	0	1	0	1	0	2	+	+
Liver, hepatocellular hypertrophy	0	0	0	0	10	3	10	3	+	+
Pancreas, increased vacuolation	0	0	0	0	0	0	3	0	+	–
Kidney, tubular cell swelling	0	0	0	0	0	0	7	3	+	+
Kidney, tubular cell necrosis*	0	0	0	0	0	0	3	0	+	–

[a] number of animals affected

*inserted by the RMS

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1994): 13-Week Oral Toxicity (Feeding) Study with [redacted] in the Hamster. [redacted] Project No. [redacted] date: 1994-10-27 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline 408 (1981)
2.2	**GLP**	Yes
2.3	**Deviations**	Deviation from current version of OECD 408: No functional observations on neurobehavioural effects were conducted.

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Fine crystalline powder, whitish
3.1.2.2	Purity	99.5%
3.1.2.3	Stability	Expiration date: December 1999 Stability in feed was verified by continued analysis
3.2	**Test Animals**	
3.2.1	Species	Syrian Golden Hamster
3.2.2	Strain	Bio-F1D, Alexander, hybrid
3.2.3	Source	[redacted]
3.2.4	Sex	Males & females
3.2.5	Age/weight at study initiation	Age: 5 weeks (at delivery) Weight range: 52-83 g (♂) and 51-80 g (♀).
3.2.6	Number of animals per group	15/sex/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	13 weeks with 7-week interim sacrifice
3.3.2	Frequency of exposure	*ad libitum*
3.3.3	Post-exposure period	none

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

3.3.4	**Oral**	
3.3.4.1	Type	In food
3.3.4.2	Doses	75, 200, 350, 750, 900 mg/kg bw/day
3.3.4.3	Vehicle	Diet
3.3.4.4	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily
3.4.1.2	Mortality	Yes, twice daily
3.4.2	Body weight	Yes, once weekly.
3.4.3	Food consumption	Yes, once weekly.
3.4.4	Water consumption	Yes, once weekly.
3.4.5	Ophthalmoscopic examination	Yes, at pre-test and at 13 weeks
3.4.6	Haematology	Yes, at pre-test and after 7 and 13 weeks Parameters: erythrocyte count + morphology, nucleated erythrocytes, haemoglobin concentration, haematocrit, MCV, MCH, MCHC, leukocyte count, differential leukocyte count, platelet count, reticulocyte count + fluorescence ratio, thromboplastin time, activated partial thromboplastin time
3.4.7	Clinical chemistry	Yes, at pre-test and after 7 and 13 weeks Parameters: sodium, potassium, calcium, chloride inorganic phosphate, glucose, urea, creatinine, total bilirubin, total cholesterol, triglycerides, total protein, albumin, globulins, A/G ratio, creatine kinase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), creatine kinase (CK), lactate dehydrogenase (LDH), γ-glutamyl-transferase (γ-GT)
3.4.8	Urinalysis	Yes, at pre-test and after 7 and 13 weeks Parameters: volume, spec. gravity, osmolality, colour, appearance, glucose, pH, protein, ketones, bilirubin, blood, urobilinogen, sediment
3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes Organs: adrenals, brain, heart, liver, spleen, kidneys, testes, thyroid
3.5.2	Gross and histopathology	Gross pathology: all dose groups; Histopathology: Adrenal glands, aorta, bone - sternum, bone marrow - sternal, brain, epididymides, oesophagus, gallbladder, heart, kidneys, large intestine - caecum, colon and rectum, larynx, liver, lungs, lymph nodes - mesenteric and mandibular, ovaries, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicles, small intestine - duodenum, jejunum and ileum, spleen, stomach, testes, thymus, thyroid glands with parathyroid glands, trachea, urinary bladder, uterus, vagina and all gross lesions.
3.5.3	Other examinations	Immunohistochemical assessment of PCNA content was performed in liver, kidney and testes at scheduled necropsy.
3.5.4	Statistics	The following statistical methods were used to analyze the food consumption, body weights organ weights and clinical laboratory data:

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

Univariate one-way ANOVA was used to assess the significance of intergroup differences.
If the variables could be assumed to follow a normal distribution the Dunnett test (many to one t-test) based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups.
The Steel-test (many-one rank test) was applied when the data could not be assumed to follow a normal distribution.

For the ophthalmoscopy the Fisher's exact test was applied.
Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

Individual values, means, standard deviations and statistics were rounded-off before printing. For example, test statistics were calculated on the basis of exact values for means and pooled variances and then rounded off to two decimal places. Therefore two groups may display the same printed means for a given parameter yet display different test statistic values.

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

There were no clinical signs which could be attributed to the administration of triclosan.

4.1.2 Mortality

There were three unscheduled deaths during the study. One control male died during pretest after blood sampling. One male at 900 mg/kg died on treatment day 37. In this animal an intussusception and prolapse of the rectum was found at necropsy. One female at 750 mg/kg died on treatment day 74. In this animal a focus in the cerebellum was found at necropsy.

4.2 Body weight gain

Body weights and body weight gains up to and including a dose of 350 mg/kg were not affected by treatment.

In groups 5 (750 mg/kg) and 6 (900 mg/kg) a retardation of body weight development was evident for both sexes from the beginning of the treatment period up to week 9. As from week 10 of treatment the mean differences from control groups persisted up to termination amounting to about 10%/17% in ma1es/females of group 5 and 13%/22% in males/females of group 6. These findings are considered to be treatment-related.

4.3 Food consumption

Overall mean food consumption was decreased in females at 350 and 750 mg/kg and in both sexes at 900 mg/kg. For females at 350 and 750 mg/kg, the average mean food intake reached 93 and 88 % of that recorded in controls. For males/females at 900 mg/kg the respective percentages amounted to 90/87%.

In males and females of all treated groups food consumption was adversely affected during week 1/2 of treatment. At this time the differences were dose related and statistically significant. Thereafter, a statistically significant reduction in food consumption was noted for both sexes at group 6 (900 mg/kg) and in part at group 5 (750 mg/kg) up to week 6/7 of treatment As front week 7/8 food consumption was similar in all treated and untreated animals.

The course of relative food consumption was characterized by a dose-related transient reduction just after start of treatment. During the further course of the study an increase in relative food consumption was noted for males and females of group 5 (750 mg/kg) and group 6 (900 mg/kg), differences being statistically significant in males of group 5 and 6 between week 7/8 and week 12/13 and in females of group 6 from week 6/7 until

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

		week 12/13 of study These findings are considered to be treatment-related. In particular at least the early transient effect may be due to the bad palatability of the test article in diet.
4.4	**Water consumption**	Administration of triclosan at a dose of 75 mg/kg had no effect on absolute or relative water consumption. At doses of 200 mg/kg up to and including 900 mg/kg a dose-related increase in absolute and relative water consumption was observed for both sexes. When compared with the controls the mean differences over the whale treatment period were about 30% at group 3 (200 mg/kg), about 70% at group 4 (350 mg/kg), 110% (females) to 144% (males) at group 5 (750 mg/kg) and 110% (females) to 167% (males) at group 6 (900 mg/kg) These findings are considered to be test-article related.
4.5	**Ophthalmoscopic examination**	No effects.
4.6	**Blood analysis**	
4.6.1	Haematology	Slightly decreased erythrocyte count, haemoglobin concentration and haematocrit in both males and females of group 6 (900 mg/kg) and in females of group (750 mg/kg), slightly decreased MCV and MCH indices in both males and females of group 6, slightly increased platelet count in both males and females of group 6, slightly increased reticulocyte count in both males and females of group 6 and in males of group 4 (350 mg/kg), slightly increased HFR and decreased LFR reticulocyte fluorescence ratio in females of group 6, moderate microcytosis and slight poikilocytosis in both males and females of group 6, slightly prolonged thromboplastin time in both males and females of group 6 and in males of group 5, and slightly to moderately prolonged partial thromboplastin time in both males and females of group 6, and slightly in males and females of group 5. The haematological findings were considered to be treatment-related and reflect greater turnover of circulating erythrocytes. In addition, the red cell morphological features and changes in the red cell indices are early indications of a microcytic type anaemia. These findings were somewhat more obvious for animals of group 6 with only a marginal effect on group 5. Moreover, prolonged coagu1ation times observed in groups 5 and 6 suggest disturbances in the coagulation pathways. This again may be related to decreased synthesis of one or more coagulation factors produced in the liver. All other differences in the results of the haematological parameters were considered to be incidental and unrelated to the treatment (see Table A6_4_1-1).
4.6.2	Clinical chemistry	Slightly decreased glucose concentration in both males and females of groups 5 (750 mg/kg) and 6 (900 mg/kg) moderately increased urea concentration in both males and females of group 6, slightly increased creatinine concentration in males and females of group 6, slightly increased total bilirubin concentration in females of group 6, slightly increased total cholesterol concentration in both males and females of group 6, slightly to moderately increased triglyceride concentration in males and slightly to markedly in females of group 6, slightly increased ALT, LDH, and CK activity in females of group 6, slightly decreased AP activity in males and females of groups 5 and 6, slightly decreased γ-GT activity in both males and females of group 6, slightly increased calcium concentration in males of groups 4 (350 mg/kg) and 6, slightly increased phosphorus concentration in both males and females of groups 5 and 6, slightly increased sodium concentration in both males and females of groups 4, 5

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

		and 6, slightly increased chloride concentration in both males and females of groups 5 and 6, and in males of group 4, slightly increased total protein concentration in males of group 6, slight changes in some protein fractions of the protein electrophoretic pattern, characterized primarily by a decreased albumin fraction in both males and females of group 6 and in ma1es of group 4, increased alpha-1 globulin fraction in both males and females of group 6 and in males of group 4, increased alpha-2 globulin fraction in both males and females of group 6, and in males of groups 4 and 5 decreased beta globulin fraction in females of group 6, increased gamma globulin fraction in both males and females of group 6 and in males of group 4, and decreased albumin to globulin ratio in both males and females of group 6 and in males of group 4 (see Table A6_4_1-1). These findings were considered to be treatment-related and reflect clinical and physiological disturbances of liver and renal function for the animals of groups 5 and 6. All other differences in the results of clinical biochemistry parameters were considered to be incidental and unrelated to the treatment.	
4.7	**Urinalysis**	Slightly to moderately increased overnight urine output (polyuria) in both males and females of groups 4 (350 mg/kg), 5 (750 mg/kg) and 6 (900 mg/kg), slightly decreased specific gravity and osmolality in both males and females of groups 3 (200 mg/kg), 4, 5 and 6, with physiological urinary protein being not detectable in both males and females of groups 4, 5 and 6, lower bilirubin scores in males and females of groups 5 and 6 and slight to moderate increase in blood (haemoglobinuria and haematuria) in both males and females of groups 3, 4, 5 and 6 (see Table A6_4_1-1). The findings suggest renal toxicity for the animals of groups 4, 5 and 6 as indicated by the degree of polyuria, haemoglobinuria and haematuria in the animals of these groups. The lower specific gravity and osmolality obtained during these periods may not be a reflection of impaired concentrating ability but a con sequence of the increased water excretion during the test period. All other differences in the results of the urinalysis parameters were considered to be incidental and unrelated to the treatment.	
4.8	**Sacrifice and pathology**		
4.8.1	Organ weights	In females organ weights were not affected by treatment. In males increased kidney weight and kidney weight ratios were noted at group 5 (750 mg/kg) after 13 weeks and at group 6 (900 mg/kg) at 46/47 days and after 13 weeks (see Table A6_4_1-2).	
4.8.2	Gross and histopathology	<u>Macroscopic Findings</u> Morphologic alterations due to the test article were noted at necropsy in the kidneys and took the form of an increased incidence of tan discoloured and/or granulated organs in group 6 at interim sacrifice and in groups 5 and 6 at final sacrifice. <u>Microscopic Findings</u> Microscopic findings in the kidneys indicated nephrotoxicity: tubular casts, tubular basophilia and tubular dilation which occurred at a dose-related increase in incidence and severity. Inflammatory alterations in the stomach seen chiefly in groups 5 and 6 were also considered to be due to the test article An additional finding in group 6 was an increase in minor degrees of splenic haematopoesis (see Table A6_4_1-2).	X

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Triclosan was administered via feed to groups of male and female Syrian hamsters for a period of 13 weeks using target dose levels of 0, 75, 200, 350, 750 and 900 mg/kg body weight per day.

An interim sacrifice was performed after 7 weeks of treatment. The study was conducted according to OECD Guideline 408.

5.2 Results and discussion

The administration of triclosan showed no effects on mortality, clinical sign/nodules and masses, and ophthalmoscopy parameters at any dose level.

At the upper dose levels of 750 and 900 mg/kg, the kidneys were identified to be the main target organs, based on macroscopic histopathologic and clinical laboratory findings. This was evidenced by increased kidney weight and kidney weight ratios in males, and by an increase of morphologic alterations, i.e. tan discoloration and/or granulated kidneys in both sexes.

The microscopic findings indicating nephrotoxicity in the kidneys were tubular casts, tubular basophilia and tubular dilation which were increased in a dose-related fashion in incidence and severity. The results of clinical biochemistry also reflected clinical and physiological disturbances of' renal function for the animals at doses of 750 and 900 mg/kg The findings of clinical biochemistry also point at the liver to be involved, but there was no histopathologic correlate to hepatic damage caused by the treatment with triclosan.

Another target of the test article was the red blood cell: a slight degradation of erythrocytes with an increase in haemopoetic activity as indicated by the increase in reticulocytes was noted mainly in the animals of the highest dose group (900 mg/kg). The histological findings correlated with increased minimal to slight degrees of splenic haematopoesis observed in animals dosed with 900 mg/kg when compared with controls.

The histopathologic findings indicate the stomach to be a target organ because of inflammatory alterations, such as gastritis and glandular erosions which were mostly at dose levels of 750 and 900 mg/kg.

At the mid-dose level of 350 mg/kg, the microscopic findings indicative of nephrotoxicity as mentioned above for the upper dose groups were observed at minor degrees and incidences. The results of the urinalysis parameters also suggest renal toxicity in hamsters of this dose group.

At the dose level of 200 mg/kg the only effects observed were an increase of water consumption, a slight decrease in urinary specific gravity and osmolality as well as a slight haemoglobinuria.

5.3 Conclusion

5.3.1	LO(A)EL	200 mg triclosan/kg bw/day ≅ 176 mg DCPP/kg bw/day, based on the onset of nephrotoxicity indicated by polyuria	X
5.3.2	NO(A)EL	75 mg triclosan/kg bw/day ≅ 66 mg DCPP/kg bw/day	
5.3.3	Reliability	1	
5.3.4	Deficiencies	No	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 October 2011
Materials and Methods	Applicants version is acceptable
Results and discussion	4.8.2 Microscopic findings: Microscopic findings in the kidneys indicated that nephrotoxicity: tubular casts, tubular basophilia and tubular dilation which occurred at a dose-related increase in incidence and severity. Inflammatory alterations in the stomach seen chiefly in groups 5 and 6 were also considered to be due to the test article. An additional finding in group 6 was an increase in minor degrees of splenic haematopoiesis (see table A6_4_1-2). Both sexes were seen to be in the suppressed phase of the breeding cycle and most male animals showed regression of testes and epididymal oligospermia, the differences in severity of the effects between groups were not statistically significant.
Conclusion	LO(A)EL: 200 mg/kg bw/day (176 mg/kg bw/day), based on the onset of nephrotoxicity indictated by polyuria NO(A)EL: 75 mg/kg bw/day (66 mg/kg bw/day DCPP), 5.2 As the animals were in the suppressed phase of the breeding cycle throughout the study it is difficult to assess potential effects on male reproductive organs.
Reliability	1
Acceptability	Acceptable
Remarks	Table A6_4_1-1 Specific gravity and osmolality was first decreased and later increased è the arrows have been adapted.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

Table A6_4_1-1. Results of clinical chemistry, haematology and urinalysis (after 13 weeks)

Parameter	Control		75 mg/kg		200 mg/kg		350 mg/kg		750 mg/kg		900 mg/kg		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	15	15	15	15	15	15	15	15	15	15	15	15		
Haematology														
RBC count	–	–	–	–	–	–	–	–	–	↓	↓	–	+	–
Haemoglobin	–	–	–	–	–	–	–	–	–	↓	↓	↓	+	+
Haematocrit	–	–	–	–	–	–	–	–	–	↓	↓	↓	+	+
MCV	–	–	–	–	–	–	–	–	–	–	↓	↓	+	+
MCH	–	–	–	–	–	–	–	–	–	–	↓	↓	+	+
Platelets	–	–	–	–	–	–	–	–	–	–	–	↑	–	+
Reticulocytes	–	–	–	–	–	–	↑	–	–	–	↑	↑	+	+
RBC microcytosis	–	–	–	–	–	–	–	–	–	–	↑	↑	+	+
RBC poikilocytosis	–	–	–	–	–	–	–	–	–	–	↑	↑	+	+
Thromboplastin time	–	–	–	–	–	–	–	–	↑	–	↑	↑	+	+
Partial thromboplastin time	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Clinical chemistry														
Glucose	–	–	–	–	–	–	–	–	–	↓	–	↓	–	+
Total cholesterol	–	–	–	–	–	–	–	–	–	–	↑	↑	+	+
Triglycerides	–	–	–	–	–	–	–	–	–	–	↑	↑	+	+
Total bilirubin	–	–	–	–	↓	–	↓	–	↓	–	–	–	–	–
AP	–	–	–	–	–	–	–	–	↓	↓	↓	↓	+	+
ALT	–	–	–	–	–	–	–	–	–	–	–	↑	–	–
AST	–	–	–	–	–	–	–	–	–	–	–	–	–	–
LDH	–	–	–	–	–	–	–	–	–	–	–	↑	–	+
γ-GT	–	–	–	–	–	–	–	–	–	–	–	↓	–	+
Urea	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Phosphorus	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Calcium	–	–	–	–	–	–	↑	–	–	–	–	–	–	–
Sodium	–	–	–	–	–	–	–	↑	↑	↑	↑	↑	+	+
Chloride	–	–	–	–	–	–	↑	–	↑	↑	↑	↑	+	+
α1-Globulin, abs.	–	–	–	–	–	–	–	–	–	–	↓	↑	–	+
α2-Globulin, abs.	–	–	–	–	–	–	–	–	↑	–	↑	↑	+	+
γ-Globulin, abs.	–	–	–	–	–	–	–	–	–	–	↑	–	+	–
Urinalysis														
Volume	–	–	–	–	–	–	↑	↑	↑	↑	↑	↑	+	+
Spec. gravity	–	–	–	–	↓	↓	↑	↑	↑	↑	↑	↑	+	+
Osmolality	–	–	–	–	↓	↓	↑	↑	↑	↑	↑	↑	+	+
Protein	–	–	–	–	–	–	↓	↓	↓	↓	↓	↓	+	+
Bilirubin	–	–	–	–	–	–	–	–	↓	↓	↓	↓	+	+
Blood	–	–	–	–	↑	–	↑	↑	↑	↑	↑	↑	+	+

↑, ↓: statistically significant increase and decrease, respectively
– : no significant difference from control values

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1 (02) 13-week oral toxicity study in hamsters

Table A6_4_1-2. Results of repeated dose toxicity study (after 13 weeks)

Parameter	Control		75 mg/kg		200 mg/kg		350 mg/kg		750 mg/kg		900 mg/kg		Dose-response +/–	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Number of animals examined	15	15	15	15	15	15	15	15	15	15	15	15		
Body weight	–	–	–	–	–	–	–	↓	↓	↓	↓	↓	+	+
Organ weights														
Brain, abs.	–	–	–	–	–	–	–	–	↓	–	↓	↓	+	+
Brain, rel.	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Heart, abs.	–	–	–	–	↓	–	–	–	↓	↓	↓	↓	+	+
Heart, rel.	–	–	–	–	–	–	–	–	↑	↑	–	↑	–	+
Liver, abs.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Liver, rel.	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Kidneys, abs.	–	–	–	–	–	–	–	–	↑	↑	↑	–	+	–
Kidneys, rel.	–	–	–	–	–	–	–	–	↑	↑	↑	↑	+	+
Spleen, abs.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Spleen, rel.	–	–	–	–	–	–	–	–	–	↑	↑	↑	+	+
Adrenals, abs.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Adrenals, rel.	–	–	–	–	–	–	–	–	–	↑	–	↑	–	+
Gross necropsy														
Kidney, tan discolouration	0[a]	1	1	0	1	0	2	0	12	12	13	11	+	+
Histopathology														
Forestomach, gastritis	1	0	1	0	0	0	0	1	1	0	1	0	–	–
Glandular stomach, gastritis	3	2	0	0	0	1	0	1	1	0	2	2	–	–
Stomach, erosion	0	1	0	1	0	1	0	2	4	5	3	7	+	+
Kidney, proliferation pelvis	0	0	0	0	0	0	0	1	2	2	3	1	+	+
Kidney, tubular casts	5	1	5	1	0	0	11	4	15	15	15	14	+	+
Kidney, tubular basophilia	12	7	10	10	12	13	15	15	15	15	15	15	+	+
Kidney, tubular dilation	6	7	10	3	10	3	12	11	15	15	15	15	+	+
Spleen, haemopoesis	0	2	0	0	0	0	0	0	0	0	10	14	+	+

[a] number of animals affected

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1(3) 90-day oral toxicity study in dogs

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1970): 90 Days Oral Toxicity Study in Beagle Dogs with [redacted] Report [redacted] date: 1970-07-10 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	The study was performed prior to endorsement of official guidelines, but followed essentially the procedure described by OECD 409
2.2	**GLP**	No
2.3	**Deviations**	Deviations from OECD Guideline 409: – test material was not characterised – the highest dose level did not elicit any signs of toxicity – not all suggested clinical chemistry parameters were investigated – uteri and gall bladders were not weighed

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	not reported Internal records on file at [redacted] show that [redacted] was a triclosan batch with a GC purity of 99.3%.
3.2	**Test Animals**	
3.2.1	Species	Dog
3.2.2	Strain	Beagle
3.2.3	Source	[redacted]
3.2.4	Sex	Males & females
3.2.5	Age/weight at study initiation	Age: 8 months Weight range: 9.5-10.6 kg (♂) and 8.7-10.0 kg (♀).
3.2.6	Number of animals per group	4/sex/group
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Duration of treatment	90 days
3.3.2	Frequency of exposure	*ad libitum*

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1(3) 90-day oral toxicity study in dogs

3.3.3	Post-exposure period	none	
3.3.4	**Oral**		
3.3.4.1	Type	In food	
3.3.4.2	Concentration	125, 313, 625 ppm ≅ 5, 12.5, 25 mg/kg bw/day	
3.3.4.3	Vehicle	Diet	
3.3.4.4	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Observations		
3.4.1.1	Clinical signs	Yes, once daily	
3.4.1.2	Mortality	Yes, once daily	
3.4.2	Body weight	Yes, thrice weekly (Mon, Tue, Wed).	
3.4.3	Food consumption	Yes, daily.	
3.4.4	Water consumption	Yes, daily.	
3.4.5	Ophthalmoscopic examination	Yes, at pre-test and at 13 weeks	
3.4.6	Haematology	Yes, at pre-test and after 5, 9 and 13 weeks Parameters: erythrocyte count, haemoglobin content, haematocrit, leukocyte count, differential leukocyte count, platelet count, reticulocyte count, thromboplastin time, blood clotting time	
3.4.7	Clinical chemistry	Yes, at pre-test and after 5, 9 and 13 weeks Parameters: paper electrophoresis, total protein, sodium, potassium, glucose, urea (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP)	X
3.4.8	Urinalysis	Yes, at pre-test and after 5, 9 and 13 weeks Parameters: volume, spec. gravity, colour, glucose, pH, protein, ketones, haemoglobin, bilirubin, sediment	
3.5	**Sacrifice and pathology**		
3.5.1	Organ weights	Yes Organs: adrenals, brain, heart, liver, lungs, spleen, kidneys, thymus, pituitary, gonads, prostate, thyroid	
3.5.2	Gross and histopathology	Gross pathology: all dose groups; Histopathology: Adrenal glands, aorta, bone - sternum, bone marrow - sternal, brain, epididymides, oesophagus, gallbladder, heart, kidneys, large intestine - caecum, colon and rectum, larynx, liver, lungs, lymph nodes - mesenteric and mandibular, ovaries, pancreas, pituitary gland, prostate gland, sciatic nerve, seminal vesicles, small intestine - duodenum, jejunum and ileum, spleen, stomach, testes, thymus, thyroid glands with parathyroid glands, trachea, urinary bladder, uterus, vagina and all gross lesions.	
3.5.3	Other examinations	Auditory acuity test, at pre-test and at 13 weeks Liver and spleen sections were stained with Prussian blue for detection of iron deposits.	
3.5.4	Statistics	Differences between group means were analysed by Student's *t*-test.	

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1(3) 90-day oral toxicity study in dogs

4 RESULTS AND DISCUSSION

4.1	**Observations**	
4.1.1	Clinical signs	There were no clinical signs which could be attributed to the administration of triclosan.
4.1.2	Mortality	No unscheduled deaths occurred.
4.2	**Body weight gain**	No effects.
4.3	**Food consumption**	No effects.
4.4	**Water consumption**	No effects.
4.5	**Ophthalmoscopic examination**	No effects.
4.6	**Blood analysis**	
4.6.1	Haematology	No effects.
4.6.2	Clinical chemistry	No effects.
4.7	**Urinalysis**	No effects.
4.8	**Sacrifice and pathology**	
4.8.1	Organ weights	No effects.
4.8.2	Gross and histopathology	Macroscopic Findings One mid-dose female displayed a stomach ulcer (size about 3mm) which reached as far as the musculature This finding was considered incidental. Microscopic Findings No indication for treatment-related changes.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Triclosan was administered via feed to groups of 4 male and 4 female Beagle dogs for a period of 13 weeks at dietary concentrations of 125, 313 or 625 ppm. These concentrations resulted in daily doses of 5, 12.5, and 25 mg/kg bw, respectively. Control dogs received plain diet. Behaviour, outward appearance, condition of faeces, uptake of food and drinking water, development of body weight, haematology, clinical chemistry, composition of urine, ophthalmic and hearing examinations as well as macroscopic and microscopic inspection and comparison of the weight of internal organs at the autopsy were performed on all animals. The study design was generally compliant with OECD Guideline 409 with minor deviations.
5.2	**Results and discussion**	There were no treatment-related effects on any of the investigated parameters.
5.3	**Conclusion**	
5.3.1	LO(A)EL	> 25 mg triclosan/kg bw/day ≅ 22 mg DCPP/kg bw/day
5.3.2	NO(A)EL	25 mg triclosan/kg bw/day ≅ 22 mg DCPP/kg bw/day
5.3.3	Reliability	2
5.3.4	Deficiencies	The test material was not characterised. However, according to Ciba's internal records, the test material with the internal code "CH 3565" represents triclosan of a purity of 99.3%.

Section 6.4.1 Subchronic toxicity

Annex Point IIA VI.6.4.1 6.4.1(3) 90-day oral toxicity study in dogs

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 October 2011
Materials and Methods	Yes, at pre-test and after 5, 9 and 13 weeks Parameters: paper electrophoresis, total protein, sodium, potassium, glucose, urea (BUN), alanine aminotranferase (ALT), aspartate aminotransferase (AST), alkaline phosphatise (AP). The following parameters were not analysed: calcium, phosphorus, chloride, ornithine decarboxylase, gamma glutamyl transpeptidase
Results and discussion	Applicant's version is adopted
Conclusion	LO(A)EL: > 25 mg/kg bw/day (22 mg/kg bw/day DCPP) NO(A)EL: 25 mg/kg bw/day (22 mg/kg bw/day DCPP)
Reliability	2
Acceptability	Acceptable
Remarks	-

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1994): 90-Day Subchronic Dermal Toxicity Study in the Rat with Satellite Group with [redacted] Report No. [redacted] date: 1994-07-14 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, US-EPA Guideline 82-3 ≅ OECD Guideline 411
2.2	**GLP**	Yes
2.3	**Deviations**	Deviations from OECD 411: – adrenals were not weighed

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Off-white powder
3.1.2.2	Purity	not reported
3.1.2.3	Stability	Stability of triclosan in vehicle was analytically confirmed throughout the study period.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CDBR (VAF/Plus)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ and ♀
3.2.5	Age/weight at study initiation	Age: ♂: 7-8 weeks, ♀: 9-10 weeks Weight: ♂: 245.6-287.2 g, ♀: 193.5-242.2 g
3.2.6	Number of animals per group	10/sex/dose level
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	13 weeks
3.3.2	Frequency of exposure	Daily (7 days/week)

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

3.3.3	Post-exposure period	28 days high-dose satellite group (10/sex)
3.3.4	**Dermal**	
3.3.4.1	Area covered	About 10% of body surface
3.3.4.2	Occlusion	Occlusive
3.3.4.3	Vehicle	Propyleneglycol
3.3.4.4	Concentration in vehicle	0, 0.5, 2.0, 4.0% (w/v)
3.3.4.5	Total volume applied	2 mL/kg bw
3.3.4.6	Dose applied	0, 10, 40, 80 mg/kg bw
3.3.4.7	Duration of exposure	6 h/day
3.3.4.8	Removal of test substance	Yes, wiping with water and paper towel
3.3.4.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily Dermal responses were evaluated prior to dosing on Days 0, 1, and 4, and twice weekly thereafter until study termination.
3.4.1.2	Mortality	Yes, twice daily (on weekends and holidays: once daily).
3.4.2	Body weight	Yes, before treatment, then once weekly.
3.4.3	Food consumption	Yes, once weekly.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes, prior to dose initiation and the in the week prior to termination of exposure
3.4.6	Haematology	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: erythrocyte count, haemoglobin concentration, haematocrit, leukocyte count, differential leukocyte count MCH, MCHC, MCV, prothrombin time, activated partial thromboplastin time, thrombocyte count
3.4.7	Clinical chemistry	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: albumin, glucose, cholesterol, urea, total bilirubin, calcium, creatinine, total protein, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), P, Cl^-, Na^+, K^+, CO_2
3.4.8	Urinalysis	Yes, all animals prior to main study termination and all satellite animals prior to satellite termination. Parameters (semi-quantitative): colour and appearance, sediment, ketones, pH, blood, bilirubin, urobilinogen, Parameters (quantitative): glucose, volume, density, protein

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes, from all animals sacrificed at termination. Organs: brain, testes/ovaries, liver, kidneys
3.5.2	Gross and histopathology	All surviving animals were sacrificed at study termination and a gross pathological examination was performed. Histopathology: from all animals of the control and highest dose group and the satellite group Organs: adrenal, aorta (thoracic), brain (cerebrum, cerebellum, brainstem), epididymides, oesophagus, exorbital lachrymal glands, eyes, femoris muscle with sciatic nerve, heart, kidneys, large intestine (sections from colon and caecum), liver, lungs (with mainstem bronchi), mammary gland (inguinal, female only), mesenteric lymph nodes, ovaries and oviducts, pancreas, pituitary, prostate, rectum, salivary glands, seminal vesicles, skin (treated and untreated) small intestine (sections from duodenum, jejunum, ileum), spinal cord (cervical, midthoracic, lumbar), spleen, sternum with marrow, stomach, testes, thymus, thyroid with parathyroids, trachea, urinary bladder, uterus (corpus, cervix), all gross lesions
3.5.3	Other examinations	–
3.5.4	Statistics	Statistical treatment of the results was conducted where appropriate. Statistical evaluation of equality of means was done by an appropriate one way ANOVA and a test for ordered response in the dose groups. First, Bartlett's Test was performed to determine if the dose groups have equal variance. If the variances were equal, the testing was done using parametric methods, otherwise nonparametric techniques were used. For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used. If significant differences among the means were indicated, Dunnett's Test was used to determine which treatment groups differed significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model. For the nonparametric procedures the test of equality of means was performed using the Kruskal-Wallis Test. If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control. In addition to the Kruskal-Wallis Test, Jonckheere's Test for monotonic trend in the dose response was performed. Bartlett's Test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance. The statistical *t*-test was also used to compare the high dose and satellite animals data (where appropriate) to substantiate their equivalence in order to accurately evaluate the recovery effect.
3.6	**Further remarks**	–

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Clinical signs observed during the test period were minimal with the majority of animals being free of abnormalities during the study. There was a low incidence of scabs, sores, alopecia, dental abnormalities, ocular discharge, and/or abnormalities of the tail observed intermittently in one or more groups. Additionally, one 10 mg/kg female was observed as emaciated, one satellite female had a swollen limb, one satellite female had a swollen ear, one control male was observed with soft stool, and one satellite male had red material adhering to the penis, hypothermia, and pale extremities. All of these findings were incidental and were not considered to be treatment-related.

Dermal observations:

Repeated topical applications of triclosan elicited erythema and/or oedema in all treated groups (10, 40, and 80 mg/kg; and satellite recovery). Erythema was not observed in any control animal.

Erythema was first observed on Day 4 in the 40 mg/kg dose group (mid dose) and in both 80 mg groups (high dose and satellite group). Erythema ranged from no erythema to severe erythema. The severity of the erythema increased as the study progressed, and the majority of animals in the mid, high, and satellite dose groups were observed with severe erythema from Day 7 until dosing termination.

Erythema also was observed in the low dose group (10 mg/kg) but the onset of erythema did not occur until Day 21. Erythema ranged from well-defined to severe in several animals, but the frequency of severe erythema scores of the low dose group was much less than observed in the higher dose groups. Erythema was also observed in one control animal. However, this erythema was judged to be mechanically induced.

Oedema was observed sporadically in the mid, high and satellite group animals. Oedema ranged from very slight to slight and was observed primarily during the latter part of the study. Very slight oedema was observed in one low dose animal. Oedema was not observed in any control animal.

Supplemental dermal observations consisting of eschar and desquamation were observed in all treated groups. More severe signs of dermal irritation, characterized by exfoliation and atonia, were generally limited to the mid, high and satellite recovery groups. Desquamation was also observed in two control animals at a very low frequency. Eschar was observed in one control animal but this irritation was considered mechanically induced.

Dermal irritation decreased dramatically in the satellite recovery group following dosing cessation. By study termination on Day 121, only one recovery animal was observed with erythema (severe) and eschar.

4.1.2 Mortality

Five animals died prior to scheduled termination. One control male was found dead on Day 22, one 80-mg/kg male was found dead on Day 84, one satellite (80 mg/kg) male was euthanised on Day 84, one 40-mg/kg and one 80-mg/kg female were found dead on Day 7.

All mortalities were considered unrelated to the test material.

4.2 Body weight gain

No effects.

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

4.3	**Food consumption and compound intake**	No effects.
4.4	**Ophthalmoscopic examination**	No treatment-related effects.
4.5	**Blood analysis**	
4.5.1	Haematology	No treatment-related effects of toxicological significance.
4.5.2	Clinical chemistry	No treatment-related effects of toxicological significance.
4.5.3	Urinalysis	No dose-related effects.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No dose-related effects.
4.6.2	Gross and histopathology	Gross pathology: Dermal irritation (desquamation, exfoliation, and/or eschar) was the only treatment-related macroscopic finding. 20 mg/kg dose group: Epididymis of one male showed several yellow areas, which were considered to be of no toxicological relevance. 500 mg/kg dose group: 2 males and one female had a pale kidney. Histopathology: Treatment-related changes were confined to the treated areas of the skin. Microscopic examination of sections of the treated skin showed several changes, the most common of which were hyperplasia/hyperkeratosis of the epidermis, sebaceous gland hyperplasia, dermal inflammation, focal epidermal necrosis, and exudates. In the mid dose male rats (40 mg/kg dose group), there was a marginal increase in the severity of sebaceous gland hyperplasia and hyperplasia/hyperkeratosis of the epidermis, as compared with controls. The same changes were more pronounced (increased severity) in the high dose male rats (80 mg/kg), as compared with controls. Male rats from both Groups 3 and 4 also showed increased incidences of dermal inflammation, focal epidermal necrosis, and exudate formation. Examination of treated skin areas from satellite recovery males showed a return to near control levels for the incidence and/or severity of these treatment-related changes The satellite animals, however, did show increased dermal fibrosis, as compared with controls and other treatment animals. Examination of the treated areas of the skin from female rats from treatment Groups 2, 3, and 4 in general showed a spectrum of changes similar to those described for the male rats. In particular, there was a dose-related increase in the severity of sebaceous gland hyperplasia, hyperplasia/hyperkeratosis of the epidermis, dermal inflammation, and focal epidermal necrosis as well as an increased incidence of exudate formation, as compared with controls. Microscopic examination of treated skin areas from the female satellite recovery animals showed a decrease in the incidence and/or severity of these changes as compared with non-recovery animals. As with the mate animals however there was an increase in the incidence of dermal fibrosis with recovery. Although changes were found in the "treated" skin from controls (sebaceous gland hyperplasia and hyperplasia/hyperkeratosis of the epidermis), the degree of severity was less than that observed for the treated animals. The changes in the skin from the control animals were

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

considered to have been the result of the clipping, application procedures, and the wrapping of the skin.

Sections of the untreated skin examined microscopically showed an increased incidence of epidermal hyperkeratosis in the satellite male rats. In general this change was minimal and considered to be the result of the clipping procedure and possible contamination of the untreated site and not related to test material administration. Also, in the female rats, the incidence and severity of the epidermal hyperkeratosis was similar between the control, high dose, and satellite animals.

Findings associated with non-dermal tissues were considered to be isolated incidental findings unrelated to the test material.

4.7 Other –

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The objective of this study was to evaluate the toxicity of triclosan following repeated topical application when administered dermally to rats for a period of at least 90 days.

In addition, a satellite recovery group was utilized to determine the reversibility, persistence, or delayed occurrence of toxic effects for at least 28 days after treatment. The test material was applied to the clipped, unabraded dorsal surface of rats 7 days per week for a minimum of 90 days. One-hundred Sprague-Dawley rats were divided into 5 groups of 10 male and 10 female rats each. Group 1 served as the carrier control group and received propylene glycol (PPG) only. Groups 2, 3, and 4, received 10, 40, and 80 mg/kg of triclosan in PPG, respectively.

Group 5 served as a satellite recovery group. Satellite animals were treated the same as the Group 4 animals and were observed for reversibility, persistence or delayed occurrence of toxic effects for at least 28 days after treatment.

Dermal responses were evaluated prior to dosing on Days 0, 1, and 4, and. twice weekly thereafter until study termination. Clinical observations were made daily for signs of toxicity and included nature, onset, severity, and duration of toxicological signs. Body weight and food consumption were measured weekly. Ophthalmoscopic examinations were made prior to dose initiation and prior to main study termination. Haematology, serum chemistry, and urinalysis studies were performed on all animals at the main study termination and again on the satellite animals at satellite group termination. All animals from Groups 1-4 were sacrificed on Test Days 92/93 and the satellite animals were sacrificed on Test Day 121. A full macroscopic examination was performed on all animals and selected organs and tissues were collected and weighed. A range of tissues were examined microscopically.

5.2 Results and discussion

Five animals died prior to scheduled study termination. The two early deaths on Day 7 (one mid dose and one high dose female) and the two late deaths on Day 84 (one high dose and one satellite male) were attributed to the wrapping procedure. The death of the control animal on Day 22 was considered incidental.

Treatment-related effects were limited to dermal irritation. Erythema and/or oedema was observed in all treated groups at the application site (10, 40, and 80 mg/kg; and satellite recovery), but was not observed in

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

		any control animal. The frequency and severity of dermal irritation was similar in the 40 mg/kg dose group (mid dose) and in both 80 mg/kg groups (high dose and satellite group). Erythema also was observed in the low dose group (10 mg/kg) but the onset of erythema was later and the frequency of scores was much less than observed in the higher dose groups. Oedema was observed sporadically in the mid, high and satellite group animals, and even less frequently in the low dose group. Supplemental dermal observations consisting of eschar and desquamation were observed in all groups, including controls. More severe signs of dermal irritation characterized by exfoliation and atonia were generally limited to the mid, high and. satellite recovery groups. Dermal irritation decreased dramatically in the satellite recovery group following dosing cessation. By study termination on Day 121, only one recovery animal was observed with severe erythema and eschar. None of the other examined parameters displayed toxicologically significant or treatment-related differences between control and treated animals.	
5.3	**Conclusion**		
5.3.1	LO(A)EL	LOAEL > 80 mg triclosan/kg bw/day ≅ 70 mg DCPP/kg bw/day, no treatment-related systemic effects	X
5.3.2	NO(A)EL	NOAEL = 80 mg triclosan/kg bw/day ≅ 70 mg DCPP/kg bw/day	X
5.3.3	Other	–	
5.3.4	Reliability	1	
5.3.5	Deficiencies	–	

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 October 2011
Materials and Methods	Applicants version is adopted
Results and discussion	Applicants version is adopted
Conclusion	Systemic LO(A)EL: >80 mg/kg bw/day (70 mg/kg bw/day DCPP) Systemic NO(A)EL: 80 mg/kg bw/day (70 mg/kg bw/day DCPP) Treatment-related effects were limited to dermal irritation. None of the other examined parameters displayed toxicologically significant or treatment-related differences between control and treated animals. No treatment-related systemic effects were observed. A NOAEL for all effects could not be set due to dermal irritation at all dose levels. The LOEAL for all effects was 10mg/kg bw/day.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](1994): 90-Day Subchronic Dermal Toxicity Study in the Rat with Satellite Group with [redacted] Report No. 1[redacted] date: 1994-07-14 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, US-EPA Guideline 82-3 ≅ OECD Guideline 411
2.2	**GLP**	Yes
2.3	**Deviations**	Deviations from OECD 411: – adrenals were not weighed
		3 MATERIALS AND METHODS
3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Off-white powder
3.1.2.2	Purity	not reported
3.1.2.3	Stability	Stability of triclosan in vehicle was analytically confirmed throughout the study period.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Crl:CDBR (VAF/Plus)
3.2.3	Source	[redacted]
3.2.4	Sex	♂ and ♀
3.2.5	Age/weight at study initiation	Age: ♂: 7-8 weeks, ♀: 9-10 weeks Weight: ♂: 245.6-287.2 g, ♀: 193.5-242.2 g
3.2.6	Number of animals per group	10/sex/dose level
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Dermal
3.3.1	Duration of treatment	13 weeks
3.3.2	Frequency of exposure	Daily (7 days/week)

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

3.3.3	Post-exposure period	28 days high-dose satellite group (10/sex)
3.3.4	**Dermal**	
3.3.4.1	Area covered	About 10% of body surface
3.3.4.2	Occlusion	Occlusive
3.3.4.3	Vehicle	Propyleneglycol
3.3.4.4	Concentration in vehicle	0, 0.5, 2.0, 4.0% (w/v)
3.3.4.5	Total volume applied	2 mL/kg bw
3.3.4.6	Dose applied	0, 10, 40, 80 mg/kg bw
3.3.4.7	Duration of exposure	6 h/day
3.3.4.8	Removal of test substance	Yes, wiping with water and paper towel
3.3.4.9	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	Yes, once daily Dermal responses were evaluated prior to dosing on Days 0, 1, and 4, and twice weekly thereafter until study termination.
3.4.1.2	Mortality	Yes, twice daily (on weekends and holidays: once daily).
3.4.2	Body weight	Yes, before treatment, then once weekly.
3.4.3	Food consumption	Yes, once weekly.
3.4.4	Water consumption	No
3.4.5	Ophthalmoscopic examination	Yes, prior to dose initiation and the in the week prior to termination of exposure
3.4.6	Haematology	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: erythrocyte count, haemoglobin concentration, haematocrit, leukocyte count, differential leukocyte count MCH, MCHC, MCV, prothrombin time, activated partial thromboplastin time, thrombocyte count
3.4.7	Clinical chemistry	Yes, all animals at main study termination and all satellite animals prior to satellite termination. Parameters: albumin, glucose, cholesterol, urea, total bilirubin, calcium, creatinine, total protein, triglycerides, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), P, Cl^-, Na^+, K^+, CO_2
3.4.8	Urinalysis	Yes, all animals prior to main study termination and all satellite animals prior to satellite termination. Parameters (semi-quantitative): colour and appearance, sediment, ketones, pH, blood, bilirubin, urobilinogen, Parameters (quantitative): glucose, volume, density, protein

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

3.5	**Sacrifice and pathology**	
3.5.1	Organ weights	Yes, from all animals sacrificed at termination. Organs: brain, testes/ovaries, liver, kidneys
3.5.2	Gross and histopathology	All surviving animals were sacrificed at study termination and a gross pathological examination was performed. Histopathology: from all animals of the control and highest dose group and the satellite group Organs: adrenal, aorta (thoracic), brain (cerebrum, cerebellum, brainstem), epididymides, oesophagus, exorbital lachrymal glands, eyes, femoris muscle with sciatic nerve, heart, kidneys, large intestine (sections from colon and caecum), liver, lungs (with mainstem bronchi), mammary gland (inguinal, female only), mesenteric lymph nodes, ovaries and oviducts, pancreas, pituitary, prostate, rectum, salivary glands, seminal vesicles, skin (treated and untreated) small intestine (sections from duodenum, jejunum, ileum), spinal cord (cervical, midthoracic, lumbar), spleen, sternum with marrow, stomach, testes, thymus, thyroid with parathyroids, trachea, urinary bladder, uterus (corpus, cervix), all gross lesions
3.5.3	Other examinations	–
3.5.4	Statistics	Statistical treatment of the results was conducted where appropriate. Statistical evaluation of equality of means was done by an appropriate one way ANOVA and a test for ordered response in the dose groups. First, Bartlett's Test was performed to determine if the dose groups have equal variance. If the variances were equal, the testing was done using parametric methods, otherwise nonparametric techniques were used. For the parametric procedures, a standard one way ANOVA using the F distribution to assess significance was used. If significant differences among the means were indicated, Dunnett's Test was used to determine which treatment groups differed significantly from control. In addition to the ANOVA, a standard regression analysis for linear response in the dose groups was performed. The regression also tested for linear lack of fit in the model. For the nonparametric procedures the test of equality of means was performed using the Kruskal-Wallis Test. If significant differences among the means were indicated, Dunn's Summed Rank Test was used to determine which treatment groups differed significantly from the control. In addition to the Kruskal-Wallis Test, Jonckheere's Test for monotonic trend in the dose response was performed. Bartlett's Test for equal variance was conducted at the 1% level of significance. All other tests were conducted at the 5% and 1% level of significance. The statistical *t*-test was also used to compare the high dose and satellite animals data (where appropriate) to substantiate their equivalence in order to accurately evaluate the recovery effect.
3.6	**Further remarks**	–

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

4 RESULTS AND DISCUSSION

4.1 Observations

4.1.1 Clinical signs

Clinical signs observed during the test period were minimal with the majority of animals being free of abnormalities during the study. There was a low incidence of scabs, sores, alopecia, dental abnormalities, ocular discharge, and/or abnormalities of the tail observed intermittently in one or more groups. Additionally, one 10 mg/kg female was observed as emaciated, one satellite female had a swollen limb, one satellite female had a swollen ear, one control male was observed with soft stool, and one satellite male had red material adhering to the penis, hypothermia, and pale extremities. All of these findings were incidental and were not considered to be treatment-related.

Dermal observations:

Repeated topical applications of triclosan elicited erythema and/or oedema in all treated groups (10, 40, and 80 mg/kg; and satellite recovery). Erythema was not observed in any control animal.

Erythema was first observed on Day 4 in the 40 mg/kg dose group (mid dose) and in both 80 mg groups (high dose and satellite group). Erythema ranged from no erythema to severe erythema. The severity of the erythema increased as the study progressed, and the majority of animals in the mid, high, and satellite dose groups were observed with severe erythema from Day 7 until dosing termination.

Erythema also was observed in the low dose group (10 mg/kg) but the onset of erythema did not occur until Day 21. Erythema ranged from well-defined to severe in several animals, but the frequency of severe erythema scores of the low dose group was much less than observed in the higher dose groups. Erythema was also observed in one control animal. However, this erythema was judged to be mechanically induced.

Oedema was observed sporadically in the mid, high and satellite group animals. Oedema ranged from very slight to slight and was observed primarily during the latter part of the study. Very slight oedema was observed in one low dose animal. Oedema was not observed in any control animal.

Supplemental dermal observations consisting of eschar and desquamation were observed in all treated groups. More severe signs of dermal irritation, characterized by exfoliation and atonia, were generally limited to the mid, high and satellite recovery groups. Desquamation was also observed in two control animals at a very low frequency. Eschar was observed in one control animal but this irritation was considered mechanically induced.

Dermal irritation decreased dramatically in the satellite recovery group following dosing cessation. By study termination on Day 121, only one recovery animal was observed with erythema (severe) and eschar.

4.1.2 Mortality

Five animals died prior to scheduled termination. One control male was found dead on Day 22, one 80-mg/kg male was found dead on Day 84, one satellite (80 mg/kg) male was euthanised on Day 84, one 40-mg/kg and one 80-mg/kg female were found dead on Day 7.

All mortalities were considered unrelated to the test material.

4.2 Body weight gain

No effects.

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

4.3	**Food consumption and compound intake**	No effects.
4.4	**Ophthalmoscopic examination**	No treatment-related effects.
4.5	**Blood analysis**	
4.5.1	Haematology	No treatment-related effects of toxicological significance.
4.5.2	Clinical chemistry	No treatment-related effects of toxicological significance.
4.5.3	Urinalysis	No dose-related effects.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	No dose-related effects.
4.6.2	Gross and histopathology	Gross pathology: Dermal irritation (desquamation, exfoliation, and/or eschar) was the only treatment-related macroscopic finding. 20 mg/kg dose group: Epididymis of one male showed several yellow areas, which were considered to be of no toxicological relevance. 500 mg/kg dose group: 2 males and one female had a pale kidney. Histopathology: Treatment-related changes were confined to the treated areas of the skin. Microscopic examination of sections of the treated skin showed several changes, the most common of which were hyperplasia/hyperkeratosis of the epidermis, sebaceous gland hyperplasia, dermal inflammation, focal epidermal necrosis, and exudates. In the mid dose male rats (40 mg/kg dose group), there was a marginal increase in the severity of sebaceous gland hyperplasia and hyperplasia/hyperkeratosis of the epidermis, as compared with controls. The same changes were more pronounced (increased severity) in the high dose male rats (80 mg/kg), as compared with controls. Male rats from both Groups 3 and 4 also showed increased incidences of dermal inflammation, focal epidermal necrosis, and exudate formation. Examination of treated skin areas from satellite recovery males showed a return to near control levels for the incidence and/or severity of these treatment-related changes The satellite animals, however, did show increased dermal fibrosis, as compared with controls and other treatment animals. Examination of the treated areas of the skin from female rats from treatment Groups 2, 3, and 4 in general showed a spectrum of changes similar to those described for the male rats. In particular, there was a dose-related increase in the severity of sebaceous gland hyperplasia, hyperplasia/hyperkeratosis of the epidermis, dermal inflammation, and focal epidermal necrosis as well as an increased incidence of exudate formation, as compared with controls. Microscopic examination of treated skin areas from the female satellite recovery animals showed a decrease in the incidence and/or severity of these changes as compared with non-recovery animals. As with the mate animals however there was an increase in the incidence of dermal fibrosis with recovery. Although changes were found in the "treated" skin from controls (sebaceous gland hyperplasia and hyperplasia/hyperkeratosis of the epidermis), the degree of severity was less than that observed for the treated animals. The changes in the skin from the control animals were

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

considered to have been the result of the clipping, application procedures, and the wrapping of the skin.

Sections of the untreated skin examined microscopically showed an increased incidence of epidermal hyperkeratosis in the satellite male rats. In general this change was minimal and considered to be the result of the clipping procedure and possible contamination of the untreated site and not related to test material administration. Also, in the female rats, the incidence and severity of the epidermal hyperkeratosis was similar between the control, high dose, and satellite animals.

Findings associated with non-dermal tissues were considered to be isolated incidental findings unrelated to the test material.

4.7 Other –

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The objective of this study was to evaluate the toxicity of triclosan following repeated topical application when administered dermally to rats for a period of at least 90 days.

In addition, a satellite recovery group was utilized to determine the reversibility, persistence, or delayed occurrence of toxic effects for at least 28 days after treatment. The test material was applied to the clipped, unabraded dorsal surface of rats 7 days per week for a minimum of 90 days. One-hundred Sprague-Dawley rats were divided into 5 groups of 10 male and 10 female rats each. Group 1 served as the carrier control group and received propylene glycol (PPG) only. Groups 2, 3, and 4, received 10, 40, and 80 mg/kg of triclosan in PPG, respectively.

Group 5 served as a satellite recovery group. Satellite animals were treated the same as the Group 4 animals and were observed for reversibility, persistence or delayed occurrence of toxic effects for at least 28 days after treatment.

Dermal responses were evaluated prior to dosing on Days 0, 1, and 4, and. twice weekly thereafter until study termination. Clinical observations were made daily for signs of toxicity and included nature, onset, severity, and duration of toxicological signs. Body weight and food consumption were measured weekly. Ophthalmoscopic examinations were made prior to dose initiation and prior to main study termination. Haematology, serum chemistry, and urinalysis studies were performed on all animals at the main study termination and again on the satellite animals at satellite group termination. All animals from Groups 1-4 were sacrificed on Test Days 92/93 and the satellite animals were sacrificed on Test Day 121. A full macroscopic examination was performed on all animals and selected organs and tissues were collected and weighed. A range of tissues were examined microscopically.

5.2 Results and discussion

Five animals died prior to scheduled study termination. The two early deaths on Day 7 (one mid dose and one high dose female) and the two late deaths on Day 84 (one high dose and one satellite male) were attributed to the wrapping procedure. The death of the control animal on Day 22 was considered incidental.

Treatment-related effects were limited to dermal irritation. Erythema and/or oedema was observed in all treated groups at the application site (10, 40, and 80 mg/kg; and satellite recovery), but was not observed in

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

		any control animal. The frequency and severity of dermal irritation was similar in the 40 mg/kg dose group (mid dose) and in both 80 mg/kg groups (high dose and satellite group). Erythema also was observed in the low dose group (10 mg/kg) but the onset of erythema was later and the frequency of scores was much less than observed in the higher dose groups. Oedema was observed sporadically in the mid, high and satellite group animals, and even less frequently in the low dose group. Supplemental dermal observations consisting of eschar and desquamation were observed in all groups, including controls. More severe signs of dermal irritation characterized by exfoliation and atonia were generally limited to the mid, high and. satellite recovery groups. Dermal irritation decreased dramatically in the satellite recovery group following dosing cessation. By study termination on Day 121, only one recovery animal was observed with severe erythema and eschar. None of the other examined parameters displayed toxicologically significant or treatment-related differences between control and treated animals.
5.3	**Conclusion**	
5.3.1	LO(A)EL	LOAEL > 80 mg triclosan/kg bw/day ≅ 70 mg DCPP/kg bw/day, no treatment-related systemic effects
5.3.2	NO(A)EL	NOAEL = 80 mg triclosan/kg bw/day ≅ 70 mg DCPP/kg bw/day
5.3.3	Other	–
5.3.4	Reliability	1
5.3.5	Deficiencies	–

Section 6.4.2 Repeated dose toxicity

Annex Point IIA VI.6.4.2 6.4.2 90-day dermal toxicity study in rats

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13 May 2008
Materials and Methods	Applicant's version is adopted
Results and discussion	Applicant's version is adopted
Conclusion	Systemic LO(A)EL: >80 mg/kg bw/day. Systemic NO(A)El: 80 mg/kg bw/day Treatment-related effects were limited to dermal irritation. None of the other examined parameters displayed toxicologically significant or treatment-related differences between control and treated animals. No treatment-related systemic effects were observed. A NOAEL for all effects could not be set due to dermal irritation at all dose levels. LOAEL for all effects was 10 mg/kg bw/day.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1986): [redacted] – 2-Year Oral Administration to Rats [redacted] Report No [redacted] date: 1986-04-28 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 453 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	**Triclosan**	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Description	Off-white powder	
3.1.2.2	Purity	99%	
3.1.2.3	Stability	Stability and homogeneity of triclosan in diet was analytically confirmed monthly throughout the study period.	X
3.2	**Test Animals**		
3.2.1	Species	Rat	
3.2.2	Strain	Sprague-Dawley (Crl:COBS®CD® (SD) BR)	
3.2.3	Source	[redacted]	
3.2.4	Sex	♂ and ♀	
3.2.5	Age/weight at study initiation	Age: 37 days Weight: ♂: 155.5-161.1 g, ♀: 127.6-129.2 g	
3.2.6	Number of animals per group	60/sex/dose level (104 week sacrifice) 20/sex/dose level (52 week sacrifice) 5/sex/dose level (each at 13, 26, 78 week sacrifice)	
3.2.7	Control animals	Yes	
3.2.8	Replacement group	No	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	104 weeks	
3.3.2	Interim sacrifice(s)	After 13, 26, 52, and 78 weeks	
3.3.3	Final sacrifice	After 104 weeks	

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

3.3.4	Frequency of exposure	*ad libitum*	
3.3.5	Postexposure period	None	
		Oral	
3.3.6	Type	In food	
3.3.7	Concentration	0, 300, 1000, 3000, (6000, 52 weeks only) ppm ≅ 0, 12, 40, 127, (247) mg triclosan/kg bw/day for males ≅ 0, 17, 56, 190, (422) mg triclosan/kg bw/day for females	
3.3.8	Vehicle	Diet	
3.3.9	Controls	Plain diet	
3.4	**Examinations**		
3.4.1	Body weight	Yes, Before start of treatment, week 1-12: once weekly week 13-termination: once monthly	
3.4.2	Food consumption	Yes week 1-12: once weekly week 13-termination: once monthly	
3.4.3	Water consumption	Yes, once monthly	
3.4.4	Clinical signs	Yes, at least twice daily (once daily on weekends and holidays)	
3.4.5	Mortality	Yes, at least twice daily (once daily on weekends and holidays)	
3.4.6	Ophthalmoscopic examination	Yes, all rats during weeks 52 and 104	
3.4.7	Haematology	Yes,	
	No. of animals:	20 rats/sex/group	
	Time points:	Weeks 13, 26, 52, 78, 104	
	Parameters:	Differential leukocyte count, haematocrit, haemoglobin, erythrocyte count, leukocyte count, reticulocyte count, clotting time	
3.4.8	Clinical Chemistry	Yes	
	No. of animals:	10 rats/sex/group	
	Time points:	Weeks 13, 26, 52, 78, 104	
	Parameters:	Glucose, total cholesterol, triglycerides, urea, total bilirubin, total protein, albumin, albumin/globulin ratio, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma glutamyl transpeptidase	X
3.4.9	Urinalysis	Yes	
	No. of animals:	10 rats/sex/group	
	Time points:	Weeks 13, 26, 52, 78, 104	
	Parameters:	pH, protein, glucose, ketones, blood, bilirubin, spec. gravity, protein, volume	

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

3.4.10	Pathology	Yes
3.4.10.1	Gross pathology	All surviving animals at scheduled sacrifice and on all animals dying spontaneously or sacrificed moribund during the study.
3.4.10.2	Organ Weights	Yes
	from:	All surviving animals at weeks 52 and 104.
	Organs:	Brain, adrenals (in pairs) heart, kidneys (in pairs), liver, ovaries (in pairs), testicles (in pairs), spleen
3.4.11	Histopathology	Yes
	from:	After 52 weeks: all tissues from the control and 6000 ppm groups After 104 weeks: all tissues from the control and 3000 ppm groups Livers were examined from all rats; pancreas in all rats of the interim sacrifices
	Organs:	All gross lesions, all tissue masses, brain (cerebrum, cerebellum, & brainstem), pituitary, eyes/optic nerves (x2), salivary glands, thyroids, parathyroids, trachea, oesophagus, stomach (cardia, fundus, pylorus), duodenum, spinal cord (three levels), jejunum, ileum, colon, thymus, heart, aorta, lungs, liver, pancreas, kidneys (x2), adrenals (x2), spleen, sciatic nerve, rectum, caecum, lymph nodes (mesenteric), urinary bladder, gonads (♂/♀ x 2), prostate (♂), epididymides (♂ x 2), uterus (♀ horns, x 2), uterus (♀, cervix), bone harrow (sternum), skeletal muscle, skin, mammary gland (♂ and ♀), vertebra/ femur (w/ marrow)
3.4.12	Other examinations	Special analysis: A minimum of 2 mL of heparinized blood and a minimum of 0.5 g kidney, 3 g liver, 0.5 g spleen, 0.5 g heart, 1 g brain, 1 g skeletal muscle, and 1 g retroperitoneal fat were collected from all rats at the time of scheduled necropsy at weeks 13, 26, 52, 78; and 50% of the surviving rats at week 104. The tissue specimens were packed in dry ice and shipped to the sponsor for analysis of tissue levels of the test article and its metabolites.
3.5	**Statistics**	Body weight, food consumption, organ weight: First, a test for outliers and Bartlett's test for homogeneity of variance were performed. If the Bartlett's test was not significant at $p \leq 0.05$, group means were compared using Dunnett's test. If Bartlett's test was significant, Behren's *t*-test with Cochran's approximation was applied. Clinical laboratory data: All numerical data that were obtained in the course of study were submitted to Research Computing Services or Scientific Systems for storage and for generation of interim/or final reports on programs developed by the Research Statistics Section of CIBA-GEIGY. These programs routinely list individual animal data and provide summary tables, and when the design requirements are set, generate statistical analyses. These analyses are designed mainly to test each parameter for possible trends existing between treatment groups that comprise different doses of the same compound and a zero dose control. If a significant trend is found, the test procedure is applied again to the remaining treatment groups, excluding the highest dose group, and so on, in order to examine the significance of comparisons of dose groups against the control. Mortality data: The number of days on test was regarded as the true death date for animals that died or were sacrificed on a schedule. Individual mortality data were analysed by Ciba-Geigy's statistics section. The survival distributions for each group and each sex were determined using Kaplan-Meier estimates. Two-sided non-parametric tests (Gehan-Wilcoxon test and Mantel-Cox logrank test were

performed to test for differences between the survival curves of the treatment groups.

Pathology data: Incidence data were analysed separately for each sex by Fisher's exact test and for both sexes by computing the convolved probabilities.

Pathology tumour data: The general method of Peto, later justified by Lagakos, was used to evaluate possible treatment-related effects on tumour incidence. Mantel's time-adjusted trend test or Tukey's exact version of this test was used to evaluate the observed and expected incidences of tumours that were considered to have caused the animal's death. The logistic regression method of Dinse and Lagakos was used to evaluate the observed and expected incidence of tumours that were considered mortality independent.

3.6	**Further remarks**	–

4 RESULTS AND DISCUSSION

4.1	**Body weight**	Significant reductions in average body weight, relative to control means, were noted in groups treated with dietary concentrations of 6000 (males and females) and 3000 ppm (females only), but not at 1000 or 300 ppm. The percent body weight difference, relative to the control group, was greater for females than for males.
4.2	**Food consumption**	An increased feed consumption was observed in 6000- and 3000-ppm males, but not in females.
4.3	**Water consumption**	No effects.
4.4	**Clinical signs**	Alopecia, chromodacryorrhoea, sore foot and sore tail were frequently observed, particularly during the second year in all treatment and control groups. A slightly higher incidence of bradypnoea, cachexia, chromaturia, pollakiuria, ptosis, skin lesion and unkempt appearance were observed in male and female rats of the 3000 ppm group. A low incidence of haematuria was also observed mostly in males of all dose groups. The condition of phthisis in male and female animals was considered to be related to a mechanical damage from orbital bleeding. Other findings were also noted but at lower frequencies. None of these clinical signs were considered directly related to treatment with the test article.
4.5	**Mortality**	No significant compound-related effects on mortality occurred during the study. During the 2 year study period, there were 40, 42, 34, and 40 deaths or sacrifices while moribund in male Groups 1 to 4, respectively; and 41, 44, 39, and 43 deaths or sacrifices while moribund in female groups 1 to 4, respectively. In the high-dose group that was terminated after 1 year (Group 5) only one male rat died after 102 days on study.
4.6	**Ophthalmoscopic examination**	No treatment-related effects.
4.7	**Haematology**	Haematological alterations were generally slight and transient in nature and were considered biologically significant only at doses ≥ 3000 ppm. These changes included: decreased mean haemoglobin (≥ 3000 ppm) and % reticulocytes (≥ 3000 ppm).

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

4.8	**Clinical Chemistry**	Statistically significant ($p \leq 0.05$) differences between treated groups and controls were noted at various time periods. In general, the changes were slight and transient and were considered biologically significant only at doses > 3000 ppm; although statistical differences also occurred at lower doses. These changes included: increased ALT and AST (3000 ppm), BUN (≥ 300 ppm), and albumin/globulin ratio (≥ 1000 ppm); and decreased total bilirubin and triglycerides (≥ 300 ppm), glucose (≥ 3000 ppm), total serum protein and albumin (≥ 3000 ppm). (See Table A6_5-1).
4.9	**Urinalysis**	Urinalysis revealed several slight but statistically significant changes at various test periods predominantly at doses > 3000 ppm. Urinary specific gravity and pH values were either increased or decreased, with respect to the control levels, at weeks 13 and 104; whereas, urinary proteins were reduced at doses > 3000 ppm.
4.10	**Pathology**	No treatment-related gross lesions were noted in rats from any group throughout the study.
4.11	**Organ Weights**	Statistically significant changes in average organ weights were noted in treated rats. The organ weight changes were generally associated with decreased mean body weight at doses >3000 ppm; however, at week 52, males in the 3000 ppm group had decreased mean relative liver weight in comparison to the control group.
4.12	**Histopathology**	Microscopic evaluations revealed enlarged centrilobular hepatocytes containing hyaline-appearing cytoplasmic "inclusions" in the 3000- and 6000-ppm males at their scheduled sacrifice interval of 13 and 52 weeks, respectively. Hepatocellular hypertrophy was also present (but lacked statistical significance) in 2 out of 5 male rats in the 3000 ppm group at 78 weeks. These lesions were not observed in rats maintained after 78 weeks. There were no differences between treated and control groups with respect to tumour incidence at 104 weeks.
4.13	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Triclosan was administered in the diet of rats for periods of 13, 26, 52, 78 and 104 weeks at concentrations of 0, 300, 1000, or 3000 ppm. In addition, another group of rats received the highest concentration of 6000 ppm for up to 52 weeks. The achieved dose levels were 0, 12, 40, 127, (247) mg/kg bw/day for males and 0, 17, 56, 190, (422) mg/kg bw/day for females. The study was conducted according to OECD Guideline 453. Routine clinical observations, ophthalmoscopic examinations body weight and feed consumption determinations, haematology, blood biochemistry, urinalysis, organ weight, and gross and microscopic evaluations were conducted to assess chronic and carcinogenic effects of triclosan administration.
5.2	**Results and discussion**	Increased food intake was found in high-dose males. Mean body weight decreases were observed to be transient during the study except for consistent significant decreases of up to 10% in high-dose females. Sporadic changes such as slight changes in protein, glucose, bilirubin, triglyceride, and blood urea nitrogen found in the first 52 weeks of the study had disappeared by 78-104 weeks. Slight changes in erythroid parameters were sporadic. Decreases in monocytes and white blood cells in ♀ and increased clotting time in ♂ were observed at 104 weeks.

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

		Absolute adrenal weights were increased in mid-dose males; absolute brain weights were decreased in high-dose males; absolute and relative ovary weights were increased in high-dose females; absolute and relative spleen weights were decreased in mid-dose females, and relative spleen weights were decreased in high-dose females at 104 weeks. Hepatocyte hypertrophy and hepatocytic inclusions (hyaline-staining) were occasionally noted in male rats at early time points but were not seen at 104 weeks. No other histological lesions, either neoplastic or non-neoplastic, were observed that were considered to be treatment-related. Specifically, there were no treatment-related tumours, including hepatic tumours, in any of the treated rats examined histologically at 52 or 104 weeks. Animals in the additional high-dose group killed at 52 weeks (247 or 422 mg/kg bw/d in ♂ and ♀, respectively) showed similar types of toxicity as the main study animals, excepting that the severity or incidences of events were increased. No tumours were observed in these animals.
5.3	**Conclusion**	The results show that triclosan is not tumourigenic in a 2-year study in rats at doses of up to 127 mg/kg bw/d in males and 190 mg/kg bw/d in females.
5.3.1	LO(A)EL	♂: 3000 ppm (≅ 112 / 167 mg DCPP/kg bw/day (♂/♀)), based on the finding of hepatocyte changes in males.
5.3.2	NO(A)EL	1000 ppm (≅ 35 / 49 mg DCPP/kg bw/day (♂/♀))
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 October 2011
Materials and Methods	3.1.2.3 Test material: Stability and homogeneity of triclosan in diet was analytically confirmed monthly throughout the study period. The expiry data of the batch was not stated. 3.4.8 Clinical chemistry: Glucose, total cholesterol, triglycerides, urea, total bilirubin, total protein, albumin, albumin/globulin ratio, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatise, gamma glutamyl transpeptidase. Ornithine decarboxylase was not analysed.
Results and discussion	4.7 Significant dose related decrease in WBC count in 3000ppm females at 104 weeks and a decrease in monocytes in 3000ppm males at 104 weeks. Clotting time was dose dependently increased in 3000ppm males at 104 weeks and in 6000ppm males at all measured timepoints.
Conclusion	LO(A)EL: 3000ppm: ♂: 112 mg/kg bw/day DCPP, ♀: 167mg/kg bw/day) for reduced WBC counts in ♀ and increased glotting time/decreased monocyte count in ♂ The applicant's version is adopted
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

Table A6_7-1. Table for clinical chemistry, haematology and urinalysis

Parameter changed	Controls		300 ppm		1000 ppm		3000 ppm		6000 ppm		Dose-related	
	♂	♀	♂	♀	♂	♂	♂	♀	♂	♀	♂	♀
Clotting time												
13 weeks	–	–	–	–	–	–	–	–	↑	–	+	–
26 weeks	–	–	–	–	–	–	↑	–	↑	–	+	–
52 weeks	–	–	↑	–	↑	–	↑	–	↑	–	–	–
78 weeks	–	–	–	–	–	–	–	–	/	/	–	–
104 weeks	–	–	–	–	–	–	↑	–	/	/	+	–
WBC count												
104 weeks	–	–	–	–	–	–	–	↓	/	/	–	+
Monocytes												
13 weeks	–	–	–	–	–	↓	–	↓	–	↓	–	–
26 weeks	–	–	–	–	–	↓	–	↓	–	↓	–	–
104 weeks	–	–	–	–	–	–	↓	–	/	/	+	–
Bilirubin												
13 weeks	–	–	↓	–	↓	–	↓	–	↓	↓	+	+
26 weeks	–	–	–	↓	–	↓	–	↓	–	↓	–	–
52 weeks	–	–	–	–	–	–	–	↓	↓	↓	+	+
104 weeks	–	–	–	–	–	–	–	–	/	/	–	–
Triglycerides												
13 weeks	–	–	–	↓	–	↓	–	↓	–	↓	–	+
26 weeks	–	–	–	–	–	–	–	–	↓	↓	+	+
52 weeks	–	–	–	–	–	–	–	–	–	↓	–	+
104 weeks	–	–	–	–	–	–	–	–	/	/	–	–
ALT (GPT)												
13 weeks	–	–	–	↓	–	↓	–	↓	–	↓	–	+
26 weeks	–	–	–	–	–	–	–	–	–	↓	–	+
52 weeks	–	–	–	–	–	–	–	–	–	↓	–	+
78 weeks	–	–	–	–	–	–	↑	–	/	/	+	–
104 weeks	–	–	–	–	–	–	–	–	/	/	–	–
AST (GOT)												
13 weeks	–	–	↓	–	↓	–	↓	–	↓	↓	+	+
52 weeks	–	–	–	–	–	–	↓	–	↓	–	+	–
78 weeks	–	–	–	–	–	–	↑	–	/	/	+	–
104 weeks	–	–	–	–	–	↓	–	↓	/	/	–	–
BUN												
13 weeks	–	–	–	–	–	–	–	↑	–	↑	–	+
26 weeks	–	–	–	–	–	–	–	–	–	↑	–	+
52 weeks	–	–	–	↑	–	↑	–	↑	↓	↑	–	–
78 weeks	–	–	–	–	–	–	–	–	/	/		–
104 weeks	–	–	–	–	–	–	–	↓	/	/		–
Urine, specific gravity												
104 weeks	–	–	–	–	–	–	–	↓	/	/	–	+

Section A6.5 / A6.7 Chronic Toxicity / Carcinogenicity

Annex Point IIA VI.6.5 / 6.7 6.5 / 6.7 Combined chronic toxicity / carcinogenicity study in the rat

Table A6_7-2. Results of chronic toxicity / carcinogenicity study

Parameter changed	Controls		300 ppm		1000 ppm		3000 ppm		6000 ppm		Dose-related	
	♂	♀	♂	♀	♂	♂	♂	♀	♂	♀	♂	♀
52-wk mortality [%]	0	0	0	0	0	0	0	10	5	0	–	–
104-wk mortality [%]	63.3	66.7	70.0	68.3	53.3	65.0	63.3	70.0	/	/	–	–
52-wk bw	–	–	–	–	–	–	–	↓	↓	↓	+	+
104-wk bw	–	–	–	–	–	–	–	–	/	/	–	–
Liver weight, relative												
52 weeks	–	–	–	–	–	–	↓	–	–	–	–	–
Liver, hepatocytes with inclusion bodies												
13 weeks	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	4/5	0/5	+	–
52 weeks	0/20	0/20	0/10	0/10	0/10	0/10	0/10	0/10	4/20	0/20	+	–
Liver, hepatocellular hypertrophy												
13 weeks	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	5/5	0/5	+	–
52 weeks	0/20	0/20	0/10	0/10	0/10	0/10	0/10	0/10	12/20	0/20	+	–

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1999): [redacted] Potential tumourigenic and chronic toxicity effects in prolonged dietary administration to hamsters. [redacted] Report No. [redacted] date: 1999-03-30 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD 451 (1981)	
2.2	**GLP**	Yes	
2.3	**Deviations**	In addition to the examinations required by OECD 451, chronic toxicity examinations were also conducted.	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in Section 2 of dossier.	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in Section 2 of dossier.	
3.1.2.1	Description	White crystalline powder	
3.1.2.2	Purity	99.5%	
3.1.2.3	Stability	Expiry date: September 2000 Stability and homogeneity of triclosan in diet was analytically confirmed throughout the study period.	
3.2	**Test Animals**		
3.2.1	Species	Syrian Golden Hamster	
3.2.2	Strain	Bio-F1D, Alexander, hybrid	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males & females	
3.2.5	Age/weight at study initiation	Age: 8 weeks Weight range: 84-133 g (♂) and 81-118 g (♀).	
3.2.6	Number of animals per group	60/sex/group (main study) 10/sex/group (interim sacrifice)	
3.2.7	Control animals	Yes, two independent control groups with 60 animals each	
3.2.8	Replacement group	Yes	
3.3	**Administration/ Exposure**	Oral	
3.3.1	Duration of treatment	Following a total acclimatisation period of approximately 3 weeks, treatment of all surviving animals continued until survival reached 40% in both control groups, at which time all surviving hamsters of that sex were terminated. The female sex was terminated in Week 91, on attaining 38% survival in Group 2 and 40% survival in Group 1 during	

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

		Week 90. However, due to the increased mortality and the poor clinical condition of the male high dose group, the criteria for termination were amended. Following consultation between Huntingdon, the Sponsor and the Regulatory Authority, the decision was taken to terminate the male sex early when a minimum of 20 animals was left alive in any dose group. This decision was made to ensure a meaningful scientific comparison of the terminal data could be made between all treated and control groups. Thus, the male sex was terminated in Week 96 when survival had reached 21 animals among the high dose males in Week 95 of treatment as a result of their rapidly deteriorating clinical condition. The study duration is, therefore, reported as 90 weeks for females and 95 weeks for males.
3.3.2	Interim sacrifice(s)	After 52 weeks
3.3.3	Final sacrifice	♂: 95 weeks, ♀: 90 weeks
3.3.4	Frequency of exposure	*ad libitum*
3.3.5	Postexposure period	None
		Oral
3.3.6	Type	In food
3.3.7	Doses	12.5, 75, 250 mg triclosan/kg bw/day
3.3.8	Vehicle	Diet
3.3.9	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Body weight	Yes, weekly
3.4.2	Food consumption	Yes, weekly
3.4.3	Water consumption	Yes, in 13-week intervals
3.4.4	Clinical signs	Yes, once daily
3.4.5	Mortality	Yes, twice daily (once daily on weekends and holidays)
3.4.6	Ophthalmoscopic examination	No
3.4.7	Haematology	Yes
	No. of animals:	10/sex/group
	Time points:	Week 50 and prior to termination
	Parameters:	Differential leukocyte count, haematocrit, haemoglobin, MCV, MCHC erythrocyte count, leukocyte count, platelet count, reticulocyte count, prothrombin time, activated partial thromboplastin time
3.4.8	Clinical Chemistry	Yes
	No. of animals:	10/sex/group
	Time points:	Week 50 and prior to termination
	Parameters:	Glucose, chloride, phosphorus, calcium, potassium, sodium, total cholesterol, triglycerides, urea, total bilirubin, total protein, albumin, globulins, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase
3.4.9	Urinalysis	Yes

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

	No. of animals:	10 rats/sex/group
	Time points:	Weeks 13, 26, 52, 78, 104
	Parameters:	pH, protein, glucose, sodium, potassium, chloride, total reducing substances, ketones, blood, bile pigments, urobilinogen, haem pigments, spec. gravity, volume, sediment
3.4.10	Pathology	Yes
3.4.10.1	Gross pathology	All surviving animals at scheduled sacrifices and on all animals dying spontaneously or sacrificed moribund during the study.
3.4.10.2	Organ Weights	Yes
	from:	All surviving animals at scheduled sacrifices and on all animals dying spontaneously or sacrificed moribund during the study.
	Organs:	Adrenals, lungs, spleen, brain, ovaries, testes (with epididymides), heart, pituitary, thymus, kidneys, prostate, thyroid, liver, salivary gland, uterus, seminal vesicles
3.4.11	Histopathology	Yes
	from:	Control and high-dose animals at interim and final sacrifie or dying spontaneously or sacrificed moribund during the study.
	Organs:	Adrenals, alimentary tract (oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon and rectum), brain (medullary, cerebellar and cortical sections), eyes, femur (with joint, bone and marrow), gall bladder, nasal cavity lachrymal gland), heart, kidneys, larynx, liver, lungs (all lobes and mainstem bronchi), lymph nodes (cervical and mesenteric), mammary gland, ovaries, other macroscopically abnormal tissue, pancreas, pituitary, prostate, salivary gland, sciatic nerve, seminal vesicles, skeletal muscle , skin, spinal column (to preserve and examine a sample of spinal cord from cervical level), spleen, sternum (for bone and marrow), testes (with epidymides), thymus (where present), thyroid (with parathyroid), tongue, trachea, urinary bladder, uterus (corpus and cervix), vagina
3.4.12	Other examinations	<u>Toxicokinetic sampling</u> Blood samples for test article analysis were obtained from all animals in the Satellite groups at the interim kill, and from 10 male and 10 female hamsters from each surviving Main group at termination. Blood samples of 2.5 mL were obtained prior to termination from Satellite and selected Main group animals. The blood was collected from the orbital sinus under light anaesthesia into tubes containing heparin anticoagulant. The tubes containing the blood samples were kept chilled after collection, the blood samples were centrifuged and the resulting plasma was removed into new tubes and kept chilled during transfer for analysis. The samples were analysed by Huntingdon Life Sciences Department of Bioanalysis, Metabolism and Pharmacokinetic Group for the levels of total compound in the plasma and any remaining plasma was stored at -70°C for possible future examination of metabolite plasma levels.

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

3.5 Statistics

The following sequence of statistical tests were used for food consumption, water consumption, bodyweight, organ weight and clinical pathology data:

If the data consisted predominantly of one particular value (relative frequency of the mode exceeds 75%), the proportion of animals with values different from the mode were analysed according to Fisher (1950) and Mantel (1963). Otherwise:

Bartlett's test was applied to test for heterogeneity of variance between treatments (Bartlett 1937). Where significant (at the 1% level) heterogeneity was found, a logarithmic transformation was applied to see if a more stable variance structure could be obtained.

If no significant heterogeneity was detected (or if a satisfactory transformation was found), a one-way analysis of variance was carried out. Where significant heterogeneity of variance was present and could not be removed by a transformation, an analysis of ranks was used, (Kruskal Wallis 1952/3).

Analysis of variance (ANOVA) was followed by Students t-test and Williams' test (Williams 1971/2) for a dose-related response, although only the one thought most appropriate for the response pattern observed was reported. The Kruskal-Wallis analyses were followed by the non-parametric equivalents of the *t*-test and Williams' test (Shirley's test 1977).

Analysis of covariance was used in place of ANOVA in the above sequence for organ weight data. ANOVA was performed using terminal bodyweight as covariate when the within-group relationship between organ weight and bodyweight attained statistical significance at the 10% level. This was done in an attempt to allow for any differences in bodyweight which may have influenced the organ weights.

Mortality was analysed using log rank methods (Mantel 1996). For selected tumours, incidence rates were analysed according to WHO International Agency for Research on Cancer (1980) recommendations, in such cases the context of observation of the tumour was determined by the pathologist. Trend tests were used based on nominal (i.e. target) dose levels.

3.6 Further remarks –

4 RESULTS AND DISCUSSION

4.1 Body weight

Body weight gain was significantly reduced throughout the study among both sexes receiving 250 mg/kg/day, with body weights only attaining approximately 50% of the weight gain achieved by control animals. During the main growth period of the study (Weeks 3 to 13), body weight gain was also marginally reduced in both sexes receiving 75 mg/kg/day.

4.2 Food consumption

During the first three weeks of treatment, food consumption was slightly reduced, when compared with the controls, in both sexes receiving 250 mg/kg/day and for females receiving 75 mg/kg Subsequently, food consumption improved for males receiving 250 mg/ kg/day and for females receiving 75 mg/kg/day such that it was comparable with controls. Overall food consumption was only marginally reduced for females receiving 250 mg/kg/day, while it was considered to have been unaffected by treatment in the remaining male and female treated groups.

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

4.3	**Water consumption**	Generally, water consumption during Weeks 1, 13, 26, 39, 52, 65 and 78 of treatment was variable, at both the individual animal and group mean levels, among all treated arid control groups. Water consumption, overall, was increased among both sexes receiving 250 mg/kg/day in comparison with the controls.
4.4	**Clinical signs**	No clinical signs indicative of a reaction to treatment with triclosan occurred during the study, apart from a general deterioration in the condition of male hamsters receiving 250 mg/kg/day during the latter period of the study.
4.5	**Mortality**	There were two hundred and sixty five Main group and three Satellite group deaths during the treatment period. The incidence of deaths among male hamsters receiving 250 mg/kg/day was significantly increased from Week 81. Neither the incidence nor distribution of deaths was affected by treatment for the remaining male and female groups.
4.6	**Ophthalmoscopic examination**	Not conducted.
4.7	**Haematology**	Marginal disturbances of various blood parameters were apparent, mainly among the high dose level female animals at the interim and terminal investigations. In Week 50, haematocrit values were marginally reduced, together with a minor increase in MCHC values for females receiving 75 or 250 mg/kg/day. In addition reticulocyte counts were increased for both sexes at the high dose level, together with slightly reduced platelet values for both sexes receiving 75 or 250 mg/kg/day. The terminal investigation revealed reduced packed cell volume, haemoglobin and red blood cell counts among females receiving 250 mg/kg/day. Total white blood cell counts were slightly increased among hamsters receiving 250 mg/kg/day, due mainly to the raised neutrophil values in males and lymphocyte counts in females at this dose level.
4.8	**Clinical Chemistry**	The interim and terminal investigations both revealed an increase in plasma urea nitrogen values for both sexes receiving 250 mg/kg/day. At the Week 50 investigation, plasma urea nitrogen values were also increased among both sexes receiving 75 mg/kg/day, together with slightly reduced calcium values among the treated female groups. The terminal male investigation revealed slightly increased triglyceride values among hamsters receiving 75 or 250 mg/kg/day and a slight reduction in plasma AST values among the high dose level males. (See Table A6_7-1).
4.9	**Urinalysis**	Group mean urinary volume was considerably and statistically significantly increased, together with an associated decrease in specific gravity values, for both sexes receiving 250 mg/kg/day. Generally protein and pH values were decreased for both sexes receiving 250 mg/kg together with low chloride values mainly among high dose level males. Microscopic examination of urine residues revealed a slight increase in the incidence of haem pigments and erythrocytes in the urine of both sexes receiving 250 mg/kg/day. (See Table A6_7-1).

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

4.10	**Pathology**	Macroscopic examination in Week 53 revealed an increased incidence of irregular cortical scarring of the kidneys among both sexes receiving 250 mg/kg/day, and among females at this dosage at termination. In addition, a slight increase in the incidence of white nodules was detected at Week 91 in the forestomach of treated female groups. Male animals killed in Week 96 did not reveal any treatment-related findings.
4.11	**Organ Weights**	No treatment-related changes.
4.12	**Histopathology**	The principle microscopic findings among hamsters killed after completion of 52 weeks of treatment consisted of distended medullary tubules and radial areas of dilated basophilic tubules, sometimes associated with eosinophilic celloidal/fibrosis among male hamsters receiving 250 mg/kg/day. Radial areas of dilated/basophilic tubules sometimes associated with eosinophilic colloidal/fibrosis, were also observed in female hamsters receiving 250 mg/kg/day. Examination of hamsters found dead, sacrificed *in extremis* or killed after completion of the treatment period revealed the following findings: No treatment-related differences in the neoplastic findings were observed between control and treated groups. The principal non-neoplastic findings were confined to treatment-related changes observed in the kidneys, epididymides, testes and stomach of hamsters receiving 250 mg/kg/day. These changes consisted of increased incidences of: Kidneys: Nephropathy in male and female hamsters. Epididymides: Abnormal spermatogenic cells, spermatozoa absent and reduced numbers of spermatozoa in male hamsters. Testes: A partial depletion of one or more generations of germ cells in male hamsters. Stomach: Multifocal and focal atypical hyperplasia in the fundic region in males and focal atypical hyperplasia in the fundic region in one female hamster. The incidence of poor clinical condition in male hamsters, together with the incidence of kidney lesions in both sexes, were considered to be the main factors contributory to death among animals receiving 250 mg/kg/day.
4.13	**Other**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Three groups of 60 male and 60 female Bio F1D Alexander Syrian hamsters received the test material FAT 80’023/S for a period of 90 weeks for females and 95 weeks for males at dose levels of 12.5, 75 or 250 mg/kg/day. Two further groups of 60 hamsters/sex of the same strain received the untreated basal diet and acted as the control groups. In addition, a further 5 groups of 10 hamsters/sex received the test material at the same dosage levels; these satellite animals were used for blood sampling and for interim sacrifice after completion of 52 weeks of treatment. Bodyweight and food consumption data were recorded weekly and clinical signs were monitored daily for all animals. Water consumption and urinalysis assessments were performed at intervals during the study and laboratory investigations were performed prior to termination of the satellite and main group animals. At termination a full necropsy was performed on all animals, including organ weight

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

		analysis and histopathological evaluation.
5.2	**Results and discussion**	Survival was significantly decreased in males and was generally poor (38-58%) in females at 90 weeks. Body weight gain was significantly decreased in all high-dose hamsters and was accompanied by a slight (3%), but significant decrease in food consumption in high-dose females. Biochemical changes observed at termination included increases (<50%) in blood urea nitrogen in high-dose hamsters and in triglycerides in mid- and high-dose males. Haematological changes observed included slight (<15%), but significant decreases in erythroid parameters in mid- and high-dose animals, increased white blood cells in high-dose animals, and increased lymphocytes in high-dose females at termination. Significant observations at 52 weeks (interim) were mainly renal changes in high-dose animals. At termination, renal nephropathy was observed in animals of all dose groups, with increased incidence and severity at the high-dose level. In addition, males showed atypical hyperplasia in the stomachs (fundic region), along with spermatozoa and germ cell effects at the high dose. One high-dose female showed atypical hyperplasia in the fundic region that was considered to be treatment-related. High-dose females showed distended gastric glands and a few treated females of all doses showed benign papillomas of the non-glandular region of the stomach. Hepatic effects were few, with only rarified hepatocytes reported in a few male animals (6/60 in high dose vs. 3/121 in controls). There were no tumours considered to be treatment-related. In summary, triclosan had little to no effect in hamsters at 12 and 75 mg/kg bw/d, and toxic effects at 250 mg/kg bw/d that resulted in the general deterioration of high-dose males after Week 80.
5.3	**Conclusion**	Triclosan is not tumourigenic in hamsters at doses of up to 250 mg/kg bw/d.
5.3.1	LO(A)EL	250 mg triclosan/kg bw/day ≅ 220 mg DCPP/kg bw/day, based on nephropathy
5.3.2	NO(A)EL	75 mg triclosan/kg bw/day ≅ 66 mg DCPP/kg bw/day
5.3.3	Reliability	1
5.3.4	Deficiencies	No

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13 May 2008
Materials and Methods	*2.3* Deviations: In addition to the examinations required by OECD 451, chronic toxicity examinations were also conducted. *No ophthalmoscopic investigation was conducted.*
Results and discussion	4.12 The principle microscopic findings among hamsters killed after completion of 52 weeks of treatment, consisted of distended medullary tubules and radial areas of dilated basophilic tubules, sometimes associated with eosinophilic celloidal/fibrosis among male hamsters receiving 250 mg/kg/day. Radial areas of dilated/basophilic tubules sometimes associated with eosinophilic colloidal/fibrosis, were also observed in female hamsters receiving 250 mg/kg/day. *Abnormal spermatogenic cells were found in the epididymides in 28% of examined animals (6/21) at terminal kill and a reduced number of, or absent spermatozoa were observed in 28% of examined males (6/21) at terminal kill in the 250 mg/kg/day dose group compared to control animals were 2/39 animals had abnormal spermatozoa and 1/39 had reduced number of spermatozoa.* …. Epididymides: Abnormal spermatogenic cells *(14/39),* spermatozoa absent *(11/39)* and reduced numbers of spermatozoa *(15/39)* in male hamsters. Testes: A partial depletion of one or more generations of germ cells *(31/39)* in male hamsters. *5.2* Significant observations at 52 weeks (interim) were renal changes in high-dose animals *along with spermatozoa and germ cell effects at the high dose.*. At termination, renal nephropathy was observed in animals of all dose groups, with increased incidence and severity at the high-dose level. In addition, males showed atypical hyperplasia in the stomachs (fundic region). One high-dose female showed atypical hyperplasia in the fundic region that was considered to be treatment-related. High-dose females showed distended gastric glands and a few treated females of all doses showed benign papillomas of the non-glandular region of the stomach. *In male hamsters, significant effects were seen on spermatozoa and germ cells at the highest dose level.*
Conclusion	LO(A)EL: 250 mg/kg bw/day, based on nephropathy and effects on spermatozoa and germ cells NO(A)El: 75 mg/kg bw/day Applicant's version is adopted.
Reliability	1
Acceptability	Acceptable.
Remarks	-

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

Table A6_7-1. Table for clinical chemistry, haematology and urinalysis (terminal values)

Parameter changed	Controls		12.5 mg/kg/day		75 mg/kg/day		250 mg/kg/day		Dose-related	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Haematology										
Haematocrit	–	–	–	–	–	–	–	↓	–	+
Haemoglobin	–	–	–	–	–	–	–	↓	–	+
Erythrocytes	–	–	–	–	–	–	–	↓	–	+
MCHC	–	–	–	–	↓	–	↓	–	–	–
MCV	–	–	–	–	↑	–	↑	↓	+	+
APTT	–	–	–	↓	–	↓	–	↓	–	+
PT	–	–	↓	–	↓	–	↓	–	–	–
Leukocytes	–	–	–	–	–	–	↑	↑	+	+
Neutrophils	–	–	–	–	–	–	↑	–	+	–
Lymphocytes	–	–	–	–	–	–	–	↑	–	+
Eosinophils	–	–	–	–	–	–	–	↑	–	+
Clin. Chemistry										
Glucose	–	–	–	–	–	–	↓	–	–	+
Blood urea	–	–	–	–	–	↑	↑	↑	+	+
ALT	–	–	–	–	–	–	↓	↓	–	–
Triglycerides	–	–	–	–	↑	–	↑	–	–	–
Urinalysis										
Volume	–	–	–	–	–	–	↑	↑	+	+
Spec. gravity	–	–	–	–	–	–	↓	↓	–	+
Protein	–	–	–	–	–	–	↓	–	+	–
Chloride	–	–	–	–	–	–	–	↓	–	+

Section A6.5/6.7 Chronic toxicity / Carcinogenicity

Annex Point IIA VI. 6.5/7 6.5/6.7 Chronic toxicity/Carcinogenicity study in the hamster

Table A6_7-2. Results of chronic toxicity / carcinogenicity study (final sacrifice)

Parameter	Control		12.5 mg/kg/day		75 mg/kg/day		250 mg/kg/day		Dose-response + / –	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
No. of animals in group	120	120	60	60	60	60	60	60	–	–
Mortality [%]	32	61	25	53	20	42	65	52	+	+
Body weight gain	–	–	–	–	–	–	↓	↓	+	+
Food consumption	–	–	–	–	–	–	–	↓	–	+
Water consumption	–	–	–	–	–	–	↑	↑	+	+
Gross pathology										
Kidney, irregular cortical scarring	48/120	38/120	23/60	27/60	18/60	29/60	26/60	31/60	–	+
Forestomach, white nodules	10/120	3/120	0/60	3/60	2/60	5/60	1/60	5/60	–	+
Histopathology										
Kidney, nephropathy	79/120	40/120	35/60	26/60	36/60	19/60	56/60	50/60	+	+
Epididymides, red. spermatozoa	7/120	/	2/60	/	1/60	/	20/60	/	+	/
Epididymides, abnormal spermatogenic cells	5/120	/	4/60	/	3/60	/	20/60	/	+	/
Testes, partial depletion of germ cells	24/120	/	12/60	/	10/60	/	40/60	/	+	/
Stomach, fundic hyperplasia, focal	0/120	0/120	0/60	0/60	0/60	0/60	11/60	1/60	+	–
Stomach, fundic hyperplasia, multifocal	0/120	0/120	0/60	0/60	0/60	0/60	5/60	0/60	+	–

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] (1999), Salmonella Typhimurium and Escherichia Coli Reverse Mutation Assay with [redacted] Project No [redacted] date: 1999-02-03 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes, OECD Guideline 471 (1997) = EC Method B.13/14 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	2-Aminoanthracene was the only positive control for the activation assays, which is not recommended by OECD 471.
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Beige solid
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in solvent was confirmed.
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	*S. typhimurium:* TA 98, TA 1537, TA 100, TA 1535 *E. coli:* WP2 uvrA
3.2.2	Deficiencies / Proficiencies	*S. typhimurium:* His auxotrophy, defective DNA repair activity (Δ uvrB) and defective LPS barrier (rfa) Ampicillin resistance (pKM101, TA 98 and TA 100 only) *E. coli:* Trp auxotrophy, uvrA DNA repair deficient.
3.2.3	Metabolic activation system	S9 mix from livers of Wistar rats treated with phenobarbital and β-naphtoflavon. (3 i.p. injections, 80 mg/kg bw/day in water). Livers were prepared 24 h after the last injection.
3.2.4	Positive control	TA 98: 4-nitro-*o*-phenylenediamine (4-NOPD, -S9) TA 1537: 4-nitro-*o*-phenylenediamine (4-NOPD, -S9) TA 100: sodium azide (NaN_3, -S9) TA 1535: sodium azide (NaN_3, -S9) WP2 uvrA: methyl methane sulfonate (MMS, -S9) All strains: 2-aminoanthracene (2-AA, +S9)
3.3	**Application of test substance**	
3.3.1	Concentrations	–S9: 0.03, 0.1, 0.3, 1.0, 3.3, and 10.0 µg/plate +S9: 0.1, 0.3, 1.0, 3.3, 10.0, and 33.3 µg/plate

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

3.3.2	Way of application	Plate incorporation (Exp. I) and pre-incubation (Exp. II)
3.3.3	Pre-incubation time	60 min
3.4	**Examinations**	See tables in appendix for examinations and results.

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	
4.1.1	without S9	No
4.1.2	with S9	No
4.2	**Cytotoxicity**	Yes, at ≥ 0.1 µg/plate without S9 and at 33 µg/plate with S9

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was performed to investigate the potential of DCPP to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, TA 100, and the *Escherichia coli* strain WP2 uvrA. The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test article was tested at the following concentrations: Without S9 mix: 0.03, 0.1, 0.3, 1.0, 3.3, and 10.0 µg/plate With S9 mix: 0.1, 0.3, 1.0, 3.3, 10.0, and 33.3 µg/plate
5.2	**Results and discussion**	In the first experiment the background growth was reduced from 1.0 up to 10.0 µg/plate in strains TA 1535, and TA 1537 without S9 mix, and in strain WP2 uvrA at 10.0 µg/plate with S9 mix. In experiment II, the background growth was reduced at 1.0 µg/plate and above in all strains without S9 mix. DCPP did not cause a relevant increase in the number of revertants. Appropriate reference mutagens showed a distinct increase in revertant colonies.
5.3	**Conclusion**	DCPP is negative in the bacterial reversion test, with and without metabolic activation.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	2012-12-07
Materials and Methods	Agree with applicant´s version
Results and discussion	Agree with applicant´s version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

Table A6_6_1-1: Table for gene mutation assay - Experiment I (plate incorporation)
Average revertants per plate without S9-mix

Concentration [µg/plate]	Revertants/plate [mean of 3 plates]				
	TA 98	TA 100	WP2 uvrA	TA 1535	TA 1537
Solvent control	26	91	33	12	10
0.03	22	81	36	8	11
0.1	19	28	43	12	11
0.3	12	12	34	4	9
1.0	5	6	16	10	3
3.3	7	3	17	2	1
10.0	2	2	15	1	1
Pos. control	348	1381	619	1164	116

Table A6_6_1-2: Table for gene mutation assay - Experiment I (plate incorporation)
Average revertants per plate in tester strains TA98, TA 100, TA 1535 and TA 1537 with S9-mix (rat)

Concentration [µg/plate]	Revertants/plate [mean of 3 plates]				
	TA 98	TA 100	WP2 uvrA	TA 1535	TA 1537
Solvent control	25	80	39	12	18
0.1	21	84	41	11	18
0.3	15	93	43	12	16
1.0	19	91	35	11	20
3.3	24	86	36	16	19
10.0	15	71	31	12	14
33.0	2	13	20	2	3
Pos. control	483	370	152	261	80

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA VI.6.6.1 6.6.1 Mutagenicity testing in bacteria – Salmonella/microsome test

Table A6_6_1-3: Table for gene mutation assay - Experiment II (pre-incubation)
Average revertants per plate without S9-mix (rat)

Concentration [µg/plate]	Revertants/plate [mean of 3 plates]				
	TA 98	TA 100	WP2 uvrA	TA 1535	TA 1537
Solvent control	23	114	35	21	14
0.03	23	96	41	25	14
0.1	24	94	33	17	13
0.3	17	11	32	6	6
1.0	6	12	14	6	24
3.3	4	6	13	5	2
10.0	4	1	13	2	2
Pos. control	227	549	146	961	61

Table A6_6_1-4: Table for gene mutation assay - Experiment II (pre-incubation)
Average revertants per plate with S9-mix (rat)

Concentration [µg/plate]	Revertants/plate [mean of 3 plates]				
	TA 98	TA 100	WP2 uvrA	TA 1535	TA 1537
Solvent control	27	124	45	15	15
0.1	29	51	35	15	18
0.3	38	48	40	15	21
1.0	35	31	41	12	29
3.3	36	22	35	15	16
10.0	36	22	37	11	15
33.0	38	3	13	45	9
Pos. control	262	564	993	142	102

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1999): *In Vitro* Chromosome Aberration Assay in Chinese Hamster V79 Cells with [redacted] date: 1999-04-22 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 473 (1997), EU Method B.10 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] = DCPP	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Description	Beige solid	
3.1.2.2	Purity	> 99%	
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in solvent was confirmed.	
3.2	**Study Type**	Chromosomal aberration assay *in vitro*	
3.2.1	Organism/cell type	Chinese Hamster V79 lung fibroblasts	
3.2.2	Metabolic activation system	S9 mix from livers of Wistar rats treated with phenobarbital and β-naphthoflavone. (3 i.p. injections, 80 mg/kg bw/day in water). Livers were prepared 24 h after the last injection.	
3.2.3	Positive control	–S9: ethylmethanesulfonate (EMS, 0.6-1.2 mg/mL) +S9: cyclophosphamide (CPA, 0.71 µg/mL)	
3.3	**Application of test substance**		
3.3.1	Concentrations	–S9: 2.5-35 µg/mL +S9: 1.25-15 µg/mL	
3.3.2	Way of application	Dissolved in DMSO	
3.3.3	Exposure time	4, 18 h	
3.4	**Examinations**		
3.4.1	Harvest times	18, 28 h	
3.4.2	Number of cells evaluated	100 per culture, 2 cultures per concentration	

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

4 RESULTS AND DISCUSSION

4.1 Genotoxicity

Ambiguous

Tables A6_6_2-3 through A6_6_2-8

4.2 Cytotoxicity

Clear toxic effects were observed after 4 h treatment with 40.63 µg/mL in the absence of S9 mix and with 20.31 µg/mL in the presence of S9 mix.

Tables A6_6_2-1 and A6_6_2-2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test article DCPP, dissolved in DMSO, was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* in three independent experiments. Following study design was employed:

	without S9 mix			with S9 mix	
	exp. I and II	exp. II	exp. III	exp. I	exp. III
Exposure period	4 h	4 h	18 h	4 h	4 h
Recovery	14 h	24 h	-	14 h	24 h
Preparation interval	18 h	28 h	18 h	18 h	28 h

In each experimental group two parallel cultures were set up. Per culture 100 metaphases were scored for structural chromosome aberrations.

The highest applied concentration in the pre-test on toxicity (2600 µg/mL) was chosen with regard to current OECD Guideline 473. Using reduced cell numbers as an indicator for toxicity, clear toxic effects were observed after 4 h treatment with 40.63 µg/mL in the absence of S9 mix and with 20.31 µg/mL in the presence of S9 mix. In addition, continuous treatment with 20.31 µg/mL and above in the absence of S9 mix induced strong toxic effects

Dose selection of the cytogenetic experiments was performed considering the toxicity data and the occurrence of precipitation.

5.2 Results and discussion

Toxic effects indicated by reduced mitotic indices were observed in the experiments II and III with and without S9 mix.

Significant increases in the number of cells carrying structural chromosomal aberrations were observed after treatment with the test article.

However, the results are ambiguous because the increase in aberration frequencies observed in Exp. I, without S9 (Table A6_6_2-3), was not apparent in the presence of S9 (Table A6_6_2-4). X

In contrast, no effect of DCPP was seen at a later harvest time (Table A6_6_2-6, Exp. II) or upon continuous treatment in the absence of S9 (Table A6_6_2-7, Exp. III). X

In contrast, continuous treatment in the presence of S9 showed a concentration-dependent increase in aberrant metaphases (Table A6_6_2-8, Exp. III). X

Because of the contradictory results from the various sets of experiments, the biological relevance of these findings is doubtful and further testing is indicated to clarify these results.

No increase in the frequencies of polyploid metaphases was found after treatment with the test article as compared to the frequencies of the

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

		controls. Appropriate mutagens were used as positive controls. They induced statistically significant increases ($p<0.05$) in cells with structural chromosome aberrations.
5.3	**Conclusion**	DCPP is ambiguous for the induction of chromosomal aberrations *in vitro*. Further *in vivo* testing is needed.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 2012-12-07

Materials and Methods State if the applicant's version is acceptable or indicate relevant discrepancies referring to the (sub) heading numbers and to applicant's summary and conclusion.

Results and discussion

5.2. Results and discussion

The discussion in 5.2. is unclear, since

- We do **not agree**, that within **experiment I** (4 hours treatment, 18h preparation interval) the **positive results without S9 mix are contradicting the negative results with S9 mix**.
- According to the summary of the study report - in **experiment II without S9 mix**, also **at the later, 28h harvest time, a significant response** was identified with 30 ug/ml, however there was no dose response in terms of statistical significance since with 35 ug/ml the result was not significantly positive. Nevertheless these data
- Treatment **for 4 hours** in the presence of S9 showed a concentration-dependent increase in aberrant metaphases (Table A6_6_2-8, Exp. III).

However we agree that the results are not fully consistent since:

- with the 4 hour treatment/18 hours harvest the dishes **without S9** mix were **positive** and the dishes **with S9** mix were **negative**.

In contrast

- with the 4 hour treatment /28 hours harvest the plates **without S9** mix did **not** show **dose-dependent positive** results and the plates **with S9** mix were **positive**.

Furthermore

- the **positive results** without S9 mix of the "**4 hours treatment**/ 18h preparation interval" were **not consistent** with the **negative results** without S9 of the "**18 hours treatment**/ 18h preparation interval"

Conclusion Under the conditions of this test, the results were positive.

Reliability 1

Acceptability acceptable

Remarks The RMS copied the summary results table from the study report to this study summary to increase clarity of the results (Table A6_6_2-9)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-1 Determination of cytotoxicity [number of cells in % solvent control], without S9 mix

Experiment I: 4 h exposure			Experiment II: 4 h exposure		
Preparation interval	Concentration* in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 h	2.5	99	18 h	5.0	**113**
"	5.0	93	"	**10.0**	95
"	10.0	74	"	**20.0**	81
"	**20.0**	84	"	25.0	78
"	**30.0**	102	"	**30.0**	103
"	40.0	28	"	35.0	20
			28 h	10.0	52
			"	20.0	50
			"	25.0	45
			"	**30.0**	37
			"	**35.0**	73

Experiment III: continuous exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 h	1.25	85
"	**2.5**	79
"	**5.0**	77
"	**10.0**	59
"	20.0	65
"	30.0	64

* rounded values

Table A6_6_2-2 Determination of cytotoxicity [number of cells in % solvent control], with S9 mix

Experiment I: 4 h exposure			Experiment III: 4 h exposure		
Preparation interval	Concentration in µg/ml	Cells in % of solvent control	Preparation interval	Concentration in µg/ml	Cells in % of solvent control
18 h	**1.25**	98	28 h	0.625	107
"	**2.5**	105	"	1.25	95
"	**5.0**	96	"	**2.5**	93
"	10.0	84	"	**5.0**	70
"	20.0	38	"	10.0	78
"	30.0	39	"	**15.0**	73

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-3 Chromosomal aberrations Experiment I, exposure 4 h, harvest time 18 h, without S9

slide no.	cells scored	% aberrant cells incl. gaps	% aberrant cells excl. gaps*	% aberrant cells with exchanges	aberrations: gaps g	gaps ig	chromatid type b	chromatid type f	chromatid type d	chromatid type ex	chromosome type ib	chromosome type if	chromosome type id	chromosome type cx	other ma	other cd
without S9 mix																
Negative control																
1	100				2	1	2	2	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	1	0	0	0	0
1 + 2	200	3.0	2.0	0.0	3	1	2	2	0	0	0	1	0	0	0	0
Solvent control: DMSO 0.5 %																
1	100				2	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	1	0	0	1	0	0	0	0	0	0
1 + 2	200	2.5	1.0	0.5	3	0	1	0	0	1	0	0	0	0	0	0
Positive control: EMS 800.0 µg / ml																
1	100				3	0	8	3	0	7	1	1	0	0	1	0
2	100				0	0	4	1	0	9	3	0	0	1	0	0
1 + 2	200	18.0	17.0	7.5	3	0	12	4	0	16	4	1	0	1	1	0
Test article: 10.0 µg / ml																
1	100				2	0	0	0	0	0	1	0	0	0	0	0
2	100				1	0	0	1	0	0	2	0	0	0	0	0
1 + 2	200	3.5	2.0	0.0	3	0	0	1	0	0	3	0	0	0	0	0
Test article: 20.0 µg / ml																
1	100				2	0	1	3	0	2	0	0	0	0	0	0
2	100				0	0	2	1	0	4	1	0	0	0	0	0
1 + 2	200	7.5	6.5	2.5	2	0	3	4	0	6	1	0	0	0	0	0
Test article: 30 µg / ml																
1	100				0	0	0	3	0	4	0	0	0	0	0	0
2	100				1	0	1	0	0	4	0	1	0	0	0	0
1 + 2	200	5.5	5.5	3.5	1	0	1	3	0	8	0	1	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-4 Chromosomal aberrations Experiment I, exposure 4 h, harvest time 18 h, with S9

slide no.	cells scored	% aberrant cells incl. gaps	% aberrant cells excl. gaps*	% aberrant cells with exchanges	aberrations: gaps g	gaps ig	chromatid type b	chromatid type f	chromatid type d	chromatid type ex	chromosome type ib	chromosome type if	chromosome type id	chromosome type cx	other ma	other cd
					with S9 mix											
Negative control																
1	100				1	0	0	1	0	0	0	1	0	0	0	0
2	100				0	0	1	0	0	0	1	0	0	0	0	0
1 + 2	200	2.5	2.0	0.0	1	0	1	1	0	0	1	1	0	0	0	0
Solvent control: DMSO 0.5 %																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	0	1	0	0	0	0	0	0	0	0
Positive control: CPA 0.71 µg /ml																
1	100				1	0	2	3	0	4	3	0	0	0	0	0
2	100				3	0	2	3	0	3	2	0	0	0	0	0
1 + 2	200	12.0	10.0	3.5	4	0	4	6	0	7	5	0	0	0	0	0
Test article: 1.25 µg / ml																
1	100				2	0	0	0	0	1	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.0	0.5	2	0	0	1	0	1	0	0	0	0	0	0
Test article: 2.5 µg /ml																
1	100				1	0	0	3	0	1	0	3	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	1.5	0.5	1	0	0	3	0	1	0	3	0	0	0	0
Test article: 5.0 µg / ml																
1	100				0	0	0	1	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	1.0	0.0	0	0	1	1	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-5 Chromosomal aberrations Experiment II, exposure 4 h, harvest time 18 h, without S9

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations: gaps g	gaps ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					without S9 mix											
Negative control																
1	100				0	0	2	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.0	1	0	2	0	0	0	0	0	0	0	0	0
Solvent control: DMSO 0.5 %																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.0	0.0	0.0	2	0	0	0	0	0	0	0	0	0	0	0
Positive control: EMS 1200.0 µg / ml																
1	100				2	0	11	1	0	25	15	2	0	0	4	0
2	100				1	0	7	4	0	17	2	1	0	0	6	0
1 + 2	200	30.0	29.5	14.5	3	0	18	5	0	42	17	3	0	0	10	0
Test article: 10.0 µg / ml																
1	100				0	0	0	0	0	5	0	0	0	0	0	0
2	100				0	0	2	0	0	1	1	0	0	1	1	0
1 + 2	200	3.0	3.0	2.5	0	0	2	0	0	6	1	0	0	1	1	0
Test article: 20.0 µg /ml																
1	100				3	0	0	1	0	3	0	0	0	0	0	0
2	100				1	1	1	0	0	3	0	0	0	0	0	0
1 + 2	200	4.0	3.5	2.5	4	1	1	1	0	6	0	0	0	0	0	0
Test article: 30.0 µg / ml																
1	100				2	0	1	1	0	1	1	0	0	0	0	0
2	100				2	0	0	0	0	0	1	1	0	0	0	0
1 + 2	200	4.0	2.0	0.5	4	0	1	1	0	1	2	1	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-6 Chromosomal aberrations Experiment II, exposure 4 h, harvest time 28 h, without S9

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with exchanges	aberrations: gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	ex	other ma	cd
					without S9 mix											
Solvent control: DMSO 0.5 %																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.0	0.0	1	0	0	0	0	0	0	0	0	0	0	0
Test article: 30.0 µg / ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				2	0	1	2	0	1	1	1	0	1	0	0
1 + 2	200	2.5	2.0	1.0	2	0	1	2	0	1	1	1	0	1	0	0
Test article: 35.0 µg / ml																
1	100				1	0	1	1	0	0	0	0	0	0	0	0
2	100				2	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	3.0	1.5	0.0	3	0	2	1	0	0	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-7 Chromosomal aberrations Experiment III, exposure 18 h, harvest time 18 h, without S9

slide no.	cells scored	% aberrant cells incl. gaps	excl. gaps*	with ex-changes	aberrations gaps g	ig	chromatid type b	f	d	ex	chromosome type ib	if	id	cx	other ma	cd
					without S9 mix											
Negative control																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	1	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.0	0	0	1	0	0	0	0	0	0	0	0	0
Solvent control: DMSO 0.5 %																
1	100				1	0	0	0	0	0	0	0	0	0	0	0
2	100				0	0	3	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.0	0.0	1	0	3	0	0	0	0	0	0	0	0	0
Positive control: EMS 600.0 µg / ml																
1	100				6	0	15	2	0	12	2	0	0	0	2	0
2	100				9	0	8	3	0	13	1	1	0	0	0	0
1 + 2	200	26.0	22.5	10.5	15	0	23	5	0	25	3	1	0	0	2	0
Test article: 2.5 µg / ml																
1	100				1	0	0	0	0	1	0	0	0	0	0	0
2	100				0	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	0.5	0.5	0.5	1	0	0	0	0	1	0	0	0	0	0	0
Test article: 5.0 µg /ml																
1	100				0	0	0	0	0	0	0	0	0	0	0	0
2	100				1	0	4	0	0	0	0	0	0	0	0	0
1 + 2	200	2.0	2.0	0.0	1	0	4	0	0	0	0	0	0	0	0	0
Test article: 10.0 µg / ml																
1	100				1	0	0	0	0	1	0	0	0	0	0	0
2	100				1	0	0	0	0	0	0	0	0	0	0	0
1 + 2	200	1.5	0.5	0.5	2	0	0	0	0	1	0	0	0	0	0	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Section A6.6.2 Genotoxicity in vitro

Annex Point IIA VI.6.6.2 6.6.2 *In-vitro* chromosome aberration assay in V79 cells

Table A6_6_2-8 Chromosomal aberrations Experiment III, exposure 4 h, harvest time 28 h, with S9

slide no.	cells scored	% aberrant cells incl. gaps	% aberrant cells excl. gaps*	% aberrant cells with ex-changes	aberrations: gaps g	gaps ig	chromatid type b	chromatid type f	chromatid type d	chromatid type ex	chromosome type ib	chromosome type if	chromosome type id	chromosome type cx	other ma	other cd
					with S9 mix											
Negative control:																
1	100				0	0	1	1	0	0	0	0	0	0	0	0
2	100				0	0	0	1	0	0	0	0	0	0	0	0
1 + 2	200	1.5	1.5	0.0	0	0	1	2	0	0	0	0	0	0	0	0
Solvent control: DMSO 0.5 %																
1	100				0	0	0	0	0	0	0	2	0	0	0	0
2	100				2	0	0	0	0	0	0	0	0	0	1	0
1 + 2	200	2.5	1.5	0.0	2	0	0	0	0	0	0	2	0	0	1	0
Positive control: CPA 0.71 µg /ml																
1	100				6	0	4	3	0	1	3	5	0	0	0	0
2	100				5	0	14	1	0	4	10	18	0	1	9	1
1 + 2	200	31.5	28.5	3.0	11	0	18	4	0	5	13	23	0	1	9	1
Test article: 2.5 µg / ml																
1	100				1	0	0	0	0	0	2	0	0	0	2	0
2	100				3	0	0	0	0	0	0	0	0	0	1	0
1 + 2	200	4.5	2.5	0.0	4	0	0	0	0	0	2	0	0	0	3	0
Test article: 5.0 µg / ml																
1	100				5	0	1	0	0	2	1	0	0	0	1	0
2	100				0	0	1	1	0	7	2	0	0	0	3	0
1 + 2	200	9.5	7.5	3.0	5	0	2	1	0	9	3	0	0	0	4	0
Test article: 15.0 µg / ml																
1	100				1	0	4	0	0	14	0	0	0	0	2	0
2	100				4	0	8	0	0	20	2	0	0	0	2	0
1 + 2	200	20.0	19.5	13.0	5	0	12	0	0	34	2	0	0	0	4	0

* inclusive cells carrying exchanges

Abbreviations

g = gap, ig = iso-gap, gaps are achromatic lesions of chromatid or chromosome type where no or only a minimal misalignment of chromosomal material is visible b = break, ib = iso-break, f = fragment, if = iso-fragment, d = deletion, id = iso-deletion, ma = multiple aberration (= more than 4 events in one cell [excluding gaps]), ex = chromatid type exchange, cx = chromosome type exchange, cd = chromosomal disintegration (= pulverization)

Table A6_6_2-9: Summary of results (copied from the study-report by RMS)

Table 1: Summary of results of the chromosomal aberration study with FAT 80´220/A

Exp.	Preparation interval	Concentration of FAT 80´220/A in µg/ml	Polyploid cells in %	Mitotic index in % of control	Aberrant cells in % incl. gaps	Aberrant cells in % excl. gaps*	Aberrant cells in % exchanges
Exposure period 4 h without S9 mix							
I	18 h	negative control	0.9	100.0	3.0	2.0	0.0
		solvent control#	0.9	100.0	2.5	1.0	0.5
		positive control###	0.9	179.9	18.0	**17.0**^{S}	7.5
		10.0	1.5	92.4	3.5	2.0	0.0
		20.0	1.3	92.8	7.5	**6.5**^{S}	2.5
		30.0	0.7	79.3	5.5	**5.5**^{S}	3.5
II	18 h	negative control	3.1	100.0	1.5	1.0	0.0
		solvent control#	2.2	100.0	1.0	0.0	0.0
		positive control####	2.8	35.6	30.0	**29.5**^{S}	14.5
		10.0	4.2	54.3	3.0	**3.0**^{S}	2.5
		20.0	5.9	60.0	4.0	**3.5**^{S}	2.5
		30.0	6.5	56.6	4.0	**2.0**^{S}	0.5
II	28 h	solvent control#	4.0	100.0	0.5	0.0	0.0
		30.0^{P}	3.8	63.6	2.5	**2.0**^{S}	1.0
		35.0^{P}	2.7	62.1	3.0	1.5	0.0
Exposure period 18 h without S9 mix							
III	18 h	negative control	4.7	100.0	0.5	0.5	0.0
		solvent control#	4.4	100.0	1.5	1.0	0.0
		positive control##	3.7	32.6	26.0	**22.5**^{S}	10.5
		2.5	4.9	68.9	0.5	0.5	0.5
		5.0	5.8	95.4	2.0	2.0	0.0
		10.0	5.5	65.6	1.5	0.5	0.5
Exposure period 4 h with S9 mix							
I	18 h	negative control	3.3	100.0	2.5	2.0	0.0
		solvent control#	3.5	100.0	0.5	0.5	0.0
		positive control§	3.2	79.0	12.0	**10.0**^{S}	3.5
		1.25	2.4	100.5	2.0	1.0	0.5
		2.5	3.3	80.8	2.0	1.5	0.5
		5.0	2.4	162.6	1.0	1.0	0.0
III	28 h	negative control	3.7	100.0	1.5	1.5	0.0
		solvent control#	5.5	100.0	2.5	1.5	0.0
		positive control§	4.3	73.9	31.5	**28.5**^{S}	3.0
		2.5	5.9	89.7	4.5	2.5	0.0
		5.0	9.3	69.8	9.5	**7.5**^{S}	3.0
		15.0	9.6	59.9	20.0	**19.5**^{S}	13.0

* inclusive cells carrying exchanges
S aberration frequency statistically significant higher than corresponding solvent control values
P precipitation occurred
\# DMSO
EMS 600 µg/ml
EMS 800 µg/ml
EMS 1200 µg/ml
§ CPA 0.71 µg/ml

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2000): Cell Mutation Assay at the Thymidine Kinase Locus ($TK^{+/-}$) in Mouse Lymphoma Cells with [redacted] Project No [redacted] date: 2000-11-30 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline 476 (1997)
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable.

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Gray to light beige solid
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in solvent was confirmed.
3.2	**Study Type**	$TK^{+/-}$ assay
3.2.1	Organism/cell type	L5178Y mouse lymphoma cells
3.2.2	Deficiencies / Proficiencies	Trifluorothymidine (TFT) sensitive, heterozygous at *tk* locus
3.2.3	Metabolic activation system	S9 mix from livers of Wistar rats treated with phenobarbital and β-naphtoflavon. (3 i.p. injections, 80 mg/kg bw/day in water). Livers were prepared 24 h after the last injection.
3.2.4	Positive control	Without S9-mix: Methylmethanesulphonate (MMS); 13 µg/mL With S9-mix: 3-Methylcholanthrene (3-MC); 3 µg/mL
3.3	**Application of test substance**	
3.3.1	Concentrations	Experiment I: –S9: 0.63 - 20 µg/mL +S9: 0.313 - 10 µg/mL Experiment II: –S9: 5.0 - 50 µg/mL
3.3.2	Way of application	The test substance was dissolved in DMSO

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

3.3.3	Pre-incubation time	4, 24 h
3.3.4	Expression time	72 h
3.4	**Examinations**	Number and size of colonies
3.4.1	Number of cells evaluated	4×10^3 per well

4 RESULTS AND DISCUSSION

4.1	**Genotoxicity**	Table A6_6_3-1 through -4
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	Strong toxic effects occurred in the first experiment at 10 and 20 µg/mL in the absence and at 5 and 10 µg/mL in the presence of metabolic activation. In the second experiment strong toxicity was observed at 20 µg/mL and above.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The study was performed to investigate the potential of DCPP to induce mutations at the mouse lymphoma thymidine kinase locus using the cell line L5178Y. The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 h. The second experiment was solely performed in the absence of metabolic activation with a treatment period of 24 hours. The highest concentration applied in the pre-test on toxicity (2600 µg/mL ≅ 10 mM) was chosen according to the molecular weight of the test item.
5.2	**Results and discussion**	Precipitation of the test item was observed at 162.5 µg/mL and above in the pre experiment. No precipitation occurred in the main experiments and the dose range was limited by toxicity. Strong toxic effects occurred in the first experiment at 10 and 20 µg/mL in the absence and at 5 and 10 µg/mL in the presence of metabolic activation. In the second experiment strong toxicity was observed at 20 µg/mL and above. The seemingly reduced toxicity in the second experiment is probably a consequence of protein binding properties of the test item. During continuous treatment for 24 h the serum concentration can not be reduced from 15 % down to 3 % as in the first experiment. In the second experiment, the high protein content of the serum may have resulted in increased concentrations of protein-bound test item and thereby reduced the toxic effects to the cells. No relevant and reproducible increase in the number of induced colonies as compared to the corresponding solvent and negative controls occurred at any test points meeting the acceptance criteria. There was also no relevant shift in the ratio of small versus large colonies.

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

		In conclusion it can be stated that during the mutagenicity test described and under the experimental conditions reported the test item did not induce mutations in the mouse lymphoma thymidine kinase locus assay using the cell line L5178Y in the absence and presence of metabolic activation. Appropriate reference mutagens to ensure sensitivity and validity of the tests were used as positive controls and showed a distinct increase in induced mutant colonies.
5.3	**Conclusion**	DCPP is considered to be non-mutagenic in this mouse lymphoma assay.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

Evaluation by Competent Authorities	
Date	2012-12-07
Materials and Methods	Agree with applicant´s version.
Results and discussion	Agree with applicant´s version.
Conclusion	Agree with applicant´s version.
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

Table A6_6_3-1: Mutagenicity data, experiment I (4 h treatment), culture I

Treatment	Concentration [µg/mL]	S9 mix	rel. total growth	Mutant colonies [per 10^6 cells]	Small colonies [per 10^6 cells]	Large colonies [per 10^6 cells]	Ratio small/large
Vehicle	–	–	100.0	100	79	20	3.95
MMS	13.0	–	46.5	420	318	102	3.12
DCPP	0.63	–	97.1	93	57	36	1.58
	1.25	–	101.9	79	53	26	2.04
	2.50	–	96.7	89	65	25	2.60
	5.00	–	55.5	76	43	33	1.30
	10.00	–	29.5	81	64	16	4.00
	20.00	–	1.6	155	103	52	1.98
Vehicle	–	+	100.0	81	60	20	3.00
3-MC	3.0	+	72.6	288	229	59	3.88
DCPP	0.31	+	102.1	98	69	29	2.38
	0.63	+	89.4	96	69	27	2.56
	1.25	+	85.1	108	79	28	2.82
	2.50	+	44.6	94	64	29	2.21
	5.00	+	37.6	98	76	22	3.45
	10.00	+	20.9	127	88	38	2.32

Table A6_6_3-2: Mutagenicity data, experiment I (4 h treatment), culture II

Treatment	Concentration [µg/mL]	S9 mix	rel. total growth	Mutant colonies [per 10^6 cells]	Small colonies [per 10^6 cells]	Large colonies [per 10^6 cells]	Ratio small/large
Vehicle	–	–	100.0	131	92	39	2.36
MMS	13.0	–	34.5	690	568	122	4.66
DCPP	0.63	–	107.6	118	79	39	2.03
	1.25	–	110.3	111	76	35	2.17
	2.50	–	110.8	144	101	44	2.30
	5.00	–	68.4	89	60	29	2.07
	10.00	–	31.0	123	94	29	3.24
	20.00	–	4.9	131	91	40	2.28
Vehicle	–	+	100.0	92	64	28	2.29
3-MC	3.0	+	58.6	274	203	72	2.82
DCPP	0.31	+	111.0	84	63	21	3.00
	0.63	+	100.6	81	59	22	2.68
	1.25	+	53.8	107	76	31	2.45
	2.50	+	43.3	101	71	30	2.37
	5.00	+	33.5	89	62	27	2.30
	10.00	+	20.4	160	103	56	1.84

Section A6.6.3 Genotoxicity in vitro

Annex Point IIA VI.6.6.3 6.6.3 Mutagenicity testing in mammalian cells – $TK^{+/-}$ assay

Table A6_6_3-3: Mutagenicity data, experiment II (24 h treatment), culture I

Treatment	Concentration [µg/mL]	S9 mix	rel. total growth	Mutant colonies [per 10^6 cells]	Small colonies [per 10^6 cells]	Large colonies [per 10^6 cells]	Ratio small/large
Vehicle	–	–	100.0	140	88	52	1.69
MMS	13.0	–	35.6	714	562	152	3.70
DCPP	5.0	–	80.9	129	101	28	3.61
	10.0	–	98.5	96	70	26	2.69
	20.0	–	54.7	89	68	21	3.24
	35.0	–	15.7	143	97	46	2.11
	50.0	–	2.9	544	403	141	2.86

Table A6_6_3-4: Mutagenicity data, experiment II (24 h treatment), culture II

Treatment	Concentration [µg/mL]	S9 mix	rel. total growth	Mutant colonies [per 10^6 cells]	Small colonies [per 10^6 cells]	Large colonies [per 10^6 cells]	Ratio small/large
Vehicle	–	–	100	120	84	36	2.33
MMS	13.0	–	35.5	801	656	145	4.52
DCPP	5.0	–	91.8	155	116	39	2.97
	10.0	–	70.9	139	100	39	2.56
	20.0	–	50.2	104	83	21	3.95
	35.0	–	14.4	191	151	40	3.78
	50.0	–	1.8	320	303	17	17.82

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 *In vivo* micronucleus assay in mouse bone marrow

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1999): Micronucleus Assay in Bone Marrow Cells of the Mouse with [redacted] Project No [redacted] date: 1999-06-09 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 474 (1997), EC Method B.12 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**		
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] = DCPP	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification		
3.1.2.1	Description	Beige solid	
3.1.2.2	Purity	> 99%	
3.1.2.3	Stability	Expiration date: October 31, 2008 Stability in solvent was confirmed.	
3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	NMRI	
3.2.3	Source	[redacted]	
3.2.4	Sex	Males and females	
3.2.5	Age/weight at study initiation	8-12 weeks old, ♂: 35.4±3.1 g, ♀: 28.2±1.7g	
3.2.6	Number of animals per group	6 per sex	
3.2.7	Control animals	Yes	
3.3	**Administration/ Exposure**		
3.3.1	Number of applications	1	
3.3.2	Interval between applications	Not applicable	
3.3.3	Post-exposure period	24, 48 h	

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 *In vivo* micronucleus assay in mouse bone marrow

		Oral
3.3.4	Type	Gavage
3.3.5	Dose	200, 670, 2000 mg/kg bw
3.3.6	Vehicle	PEG 400
3.3.7	Concentration in vehicle	20, 67, 200 mg /mL
3.3.8	Total volume applied	10 mL/kg bw
3.3.9	Substance used as positive control	Cyclophosphamide, 40 mg/kg bw
3.3.10	Controls	Vehicle and positive control
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Femoral bone marrow
	Number of animals:	All animals
	Number of cells:	2000 per animal
	Time points:	24 h, 48 h (high dose only)
	Type of cells	Erythrocytes of bone marrow
	Parameters:	Micronucleated cells, ratio of poly- to normo-chromatic erythrocytes
3.5	**Further remarks**	–

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Only reported for the dose-finding pre-test on two animals per sex. At 2000 mg/kg bw (oral gavage), all animals showed reduced activity, 2/4 showed ptosis, and 2/4 were apathic at one hour after dosage.
4.2	**Genotoxicity**	No (Table A6_6_4-1).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was performed to investigate the potential of DCPP to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. The test item was formulated in polyethylene glycol 400 (PEG 400). PEG 400 was used as vehicle control. The volume administered orally was 10 mi/kg bw. 24 h and 48 h after a single administration of the test item the bone marrow cells were collected for micronuclei analysis. Ten animals (5 males, 5 females) per test group were evaluated for the occurrence of micronuclei. 2000 polychromatic erythrocytes (PCEs) per animal were scored for micronuclei. To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes (NCE) was determined in the same sample and reported as the number of NCEs per 2000 PCEs. The following dose levels of the test item were investigated: 24 h preparation interval: 200, 670, and 2000 mg/kg bw. 48 h preparation interval: 2000 mg/kg bw. The highest dose (2000 mg/kg; maximum guideline-recommended

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 *In vivo* micronucleus assay in mouse bone marrow

		dose) was estimated by a pre-experiment to be suitable.
5.2	**Results and discussion**	After treatment with the test item the number of NCEs was not substantially increased as compared to the mean value of NCEs of the vehicle control thus indicating that DCPP had no cytotoxic effectiveness in the bone marrow. In comparison to the corresponding vehicle controls there was no enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item and with any dose level used. 40 mg/kg bw cyclophosphamide administered per os was used as positive, control which showed a substantial increase of induced micronucleus frequency.
5.3	**Conclusion**	DCPP is considered to be non-mutagenic in this micronucleus assay.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 *In vivo* micronucleus assay in mouse bone marrow

Evaluation by Competent Authorities	
Date	2012-12-07
Materials and Methods	Agree with applicant´s version
Results and discussion	Agree with applicant´s version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	acceptable
Remarks	No cytotoxicity in the bone marrow was observed, however since the test was carried out at the limit dose of 2000 mg/kg bw and clinical signs of toxicity were observed at this top dose level, the test is considered as valid.

Section A6.6.4 Genotoxicity in vivo

Annex Point IIA VI.6.6.4 6.6.4 *In vivo* micronucleus assay in mouse bone marrow

Table A6_6_4-1: Table for In Vivo Micronucleus test

test group	dose mg/kg b.w.	sampling time (h)	PCEs with micronuclei (%)	range	PCE/ NCE
vehicle	0	24	0.115	1 - 4	2000/ 1803
test item	200	24	0.075	0 - 4	2000/ 1940
test item	670	24	0.065	0 - 4	2000/ 2045
test item	2000	24	0.090	0 - 4	2000/ 1903
cyclo-phosphamide	40	24	1.480	14 - 51	2000/ 2070
test item	2000	48	0.060	0 - 3	2000/ 1979

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](2002): *In Vivo* / *In Vitro* Unscheduled DNA Synthesis in Rat Hepatocytes with [redacted] Project [redacted] date: 2002-01-30 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD Guideline 486 (1997) = EC Method B.39 (2000)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted] = DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	Gray solid
3.1.2.2	Purity	> 99%
3.1.2.3	Stability	Expiration date: October 31, 2008
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar, HanIbm:WIST(SPF)
3.2.3	Source	[redacted]
3.2.4	Sex	Males
3.2.5	Age/weight at study initiation	6-10 weeks old, mean bw: 198 ± 19.8g
3.2.6	Number of animals per group	3
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	
3.3.1	Number of applications	1
3.3.2	Interval between applications	Not applicable
3.3.3	Post-exposure period	2, 16 h
		Oral
3.3.4	Type	Gavage

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

3.3.5	Dose	500, 2000 mg/kg bw
3.3.6	Vehicle	PEG 400
3.3.7	Concentration in vehicle	50, 200 mg /mL
3.3.8	Total volume applied	10 mL/kg bw
3.3.9	Substance used as positive control	2 h preparation interval: *N,N'*-Dimethylhydrazinedihydrochloride (DMH), 40 mg/kg bw, in saline, single oral application 16 h preparation interval: 2-Acetylaminofluorene (2-AAF), 100 mg/kg bw, DMSO/PEG 400 (1+9), single oral application
3.3.10	Controls	Vehicle and positive control
3.4	**Examinations**	
3.4.1	Clinical signs	Yes
3.4.2	Tissue	Liver
	Number of animals:	3 per dose
	Number of cells:	1.3×10^8 – 5.0×10^8 cells per animal
	Time points:	2, 16 h for hepatocyte isolation 2 and 4 h for serum sampling, 3 animals per time point
	Type of cells	Hepatocytes
	Parameters:	^{3}H-incorporation (silver grain formation), serum samples

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	Only reported for the dose-finding pre-test on two animals. At 2000 mg/kg bw (oral gavage), all animals showed reduced activity 24 h after dosage.
4.2	**Genotoxicity**	No (see Tables A6_6_5-1 and -2).
4.3	**Other**	Serum levels of DCPP at 2 and 4 h after dosage ranged from 320.8 – 473.4 µg/mL.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The test item DCPP was assessed in the *in vivo / in vitro* UDS assay for its potential to induce DNA repair (UDS) in the hepatocytes of rats. The test item was formulated in PEG 400. Therefore, PEG 400 was used as vehicle control. The volume administered orally was 10 mL/kg body weight. After a treatment period of 2 and 16 hours, respectively, the animals were anaesthetized and sacrificed by liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to methyl-^{3}H-thymidine which is incorporated if UDS occurs. The test item was tested at the following dose levels: 2 and 16 hours preparation intervals: 500 and 2000 mg/kg bw. The highest dose was estimated by pre-experiments to be suitable. The bioavailability of the test item was confirmed by gas chromatography-mass spectrometry (GC-MS) analysis of serum samples taken from satellite animals 2 and 4 hours post treatment. For each experimental group inclusive the controls, hepatocytes from three treated animals were assessed for the occurrence of UDS.

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

5.2	**Results and discussion**	The viability of the hepatocytes was not substantially affected due to the in vivo treatment with the test item. No dose level of the test item revealed UDS induction in the hepatocytes of the treated animals as compared to the current vehicle controls (Table A6_6_5-1). No substantial shift to higher values was observed in the percentage distribution of nuclear grain counts (Table A6_6_5-2). Appropriate reference mutagens (DMH, 40 mg/kg bw and 2-AAF, 100 mg/kg bw) were used as positive controls. Treatment with the positive control substances revealed distinct increases in the number of nuclear and net grain counts.
5.3	**Conclusion**	DCPP is negative in the rat *in vivo / in vitro* UDS test system.
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

Evaluation by Competent Authorities	
Date	2012-12-11
Materials and Methods	Agree with applicant´s version
Results and discussion	Agree with applicant´s version
Conclusion	Agree with applicant´s version
Reliability	1
Acceptability	acceptable
Remarks	

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

Table A6_6_5-1 Mean nucleus, cytoplasmic area and net grains

Treatment	Period	Grains per nucleus		Grains per cytoplasmic area		Net grains per nucleus	
		Mean*	SD**	Mean*	SD**	Mean*	SD**
PEG 400	2 h	13.94	± 6.64	19.89	± 8.19	-5.95	± 6.64
500 mg/kg b.w. FAT 80'220/A	2 h	11.63	± 5.07	17.79	± 6.95	-6.16	± 6.41
2000 mg/kg b.w. FAT 80'220/A	2 h	12.33	± 6.15	16.14	± 8.01	-3.81	± 6.33
40 mg/kg b.w. DMH	2 h	20.62	± 10.67	15.22	± 7.85	5.40	± 6.68
PEG 400	16 h	11.73	± 5.63	19.03	± 7.89	-7.30	± 6.14
500 mg/kg b.w. FAT 80'220/A	16 h	9.95	± 4.13	17.34	± 6.59	-7.39	± 6.26
2000 mg/kg b.w. FAT 80'220/A	16 h	16.03	± 5.95	29.87	± 10.88	-13.84	± 9.79
100 mg/kg b.w. 2-AAF	16 h	49.17	± 14.46	22.27	± 7.21	26.91	± 14.97

* Mean of 3 animals
** Standard deviation

Section A6.6.5 Genotoxicity in vivo

Annex Point IIA VI.6.6.5 6.6.5 *In-vivo* unscheduled DNA synthesis in rat primary hepatocytes

Table A6_6_5-2 Percentage distribution of the nuclear grain counts

Treatment	Period	Mean*	>0	>1	>5	>10	>20	>30	>40	>50	>60	>70	>80	>90	>100
PEG 400	2 h	13.94	100	100	96.0	62.0	15.7	2.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
500 mg/kg b.w. FAT 80'220/A	2 h	11.63	100	99.0	90.0	53.3	4.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2000 mg/kg b.w. FAT 80'220/A	2 h	12.33	100	100	90.7	55.3	10.3	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
40 mg/kg b.w. DMH	2 h	20.62	100	100	95.7	78.7	48.0	16.7	5.0	1.0	0.0	0.0	0.0	0.0	0.0
PEG 400	16 h	11.73	100	99.7	90.3	52.7	4.3	1.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
500 mg/kg b.w. FAT 80'220/A	16 h	9.95	100	99.7	88.0	40.7	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
2000 mg/kg b.w. FAT 80'220/A	16 h	16.03	100	100	99.3	83.3	21.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
100 mg/kg b.w. 2-AAF	16 h	49.17	100	100	100	100	99.3	91.7	69.3	44.3	20.7	7.0	2.3	0.7	0.7

* Mean of 3 animals

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](1992a): A Segment II Teratology Study with [redacted]. [redacted] Project No. [redacted] date: 1992-04-16 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No guideline statement, but general compliance with OECD Guideline 414 in its original (1981) version
2.2	**GLP**	Yes
2.3	**Deviations**	Deviations from current version of OECD 414: – Treatment was terminated after gestation day 15. – Relative humidity in the animal facility exceeded the recommended range (recommended by OECD 414: 50-60%, actual: 22-75%).

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not reported, specification is on file at the sponsor's facility
3.1.2.1	Stability	Content, stability and homogeneity in vehicle was verified analytically
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (CD®)
3.2.3	Source	[redacted]
3.2.4	Sex	♀
3.2.5	Age/weight at study initiation	Age: 13 weeks Weight: 225.8 - 331.1 g
3.2.6	Number of animals per group	24
3.2.7	Control animals	Yes
3.2.8	Mating period	Overnight until cohabitation had occurred
3.3	**Administration/ Exposure**	**Oral**
3.3.1	Duration of exposure	Day 6-15 post mating
3.3.2	Postexposure period	Day 15 -20 post mating
3.3.3	Type	Gavage

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

3.3.4	Dose levels	15, 50, 150 mg triclosan/kg bw/day (target dose levels) 15.8, 52.3, 156.6 mg triclosan/kg bw/day (actual dose levels)
3.3.5	Vehicle	1% (w/w) carboxymethylcellulose in 20% aqueous glycerol
3.3.6	Concentration in vehicle	0.3%, 1.0%, 3.0% (w/w)
3.3.7	Total volume applied	5 mL/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Days 0, 6, 8, 10, 12, 14, 16, 18, and 20 of gestation.
3.4.2	Food consumption	Days of gestation: 0-6, 6-11, 11-16 and 16-20
3.4.3	Clinical signs	Twice daily
3.4.4	Examination of uterine content	Number of implantations, number of corpora lutea, uterus weight, number of live and death foetuses, early and late resorptions
3.4.5	Maternal organ weights	Liver and uterus w/ ovaries
3.4.6	Examination of foetuses	
3.4.6.1	General	Each foetus was individually identified, weighed, sexed externally (ano-genital distance) and given a gross examination for external mal-formations / variations to include observation for palatal defects. Late resorptions were weighed and discarded.
3.4.6.2	Soft tissue	Approximately one-half of the foetuses in each litter (alternating foetuses within the litter) were evaluated for visceral malformations/ variations using a micro-dissection procedure. Evaluations were performed on the fresh foetal specimens shortly after removal from the uterus. Foetuses designated for visceral evaluation were decapitated (head placed in appropriately labelled tissue bags and fixed Bouin's solution for later evaluation). The foetal specimens were then secured beneath a dissecting microscope (10-20X) and dissected so as to permit evaluation of tissues in the thoracic and abdominal cavities. At the completion of the foetal examination, the foetuses were eviscerated (viscera discarded) and placed in individual plastic cassettes and stored in a 70% ethanol solution. Following a period of fixation, the foetal heads were sectioned using a razor blade. The serial, transverse sections generated during this procedure were evaluated for malformations of the palate eyes and brain under a dissecting microscope (10-20X). Following evaluation, head sections were placed in plastic cassettes for storage (one litter/jar) in a 70% ethanol solution.
3.4.6.3	Skeleton	The remaining foetuses in each litter were sacrificed via an overdose of inhaled carbon dioxide. The intact foetuses were eviscerated (internally sexed by inspection of the gonads) and processed for staining of the ossified skeletal structures using the Alizarin Red S staining procedure. Foetal skeletal specimens were evaluated under a dissecting microscope (10-20X) for ossification variations and malformations.
3.4.7	Statistics	Interval data: Statistical evaluation of equality of means was made by the appropriate ore way analysis of variance technique, followed by a multiple comparison procedure if needed. First Bartlett's test was

performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control.

A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's Statistic for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1% two-sided risk level.

All ratios were transformed via Bartlett's transformation followed by the arc sine transformation prior to analysis. Data are presented untransformed.

Incidence data: Statistical analysis of incidence data was performed using contingency tables. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2x2 Fisher Exact Test; the significance level was corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Thirdly, Armitage's test for linear trend in the dosage groups was performed. In keeping with standard statistical practice, if any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

3.5 Further remarks –

4 RESULTS AND DISCUSSION

4.1	**Maternal toxic effects**	
4.1.1	Clinical signs	No effects
4.1.2	Mortality	No mortality occurred in the control group or in the low- and mid-dose group. In the high-dose group, one female died on Day 7 of gestation after two days of treatment. From necropsy findings, the death of this animal was attributed to an intubation injury and not considered treatment-related. High-dose Female No. 4575 was sorted into this group as a replacement animal.
4.1.3	Food consumption	There was a slight but significant decrease in food consumption from Days 6 through 11 of gestation at the high dose (see Table A6_8_1-1).
4.1.4	Other	Pregnancy rates, body weight development, liver weights, and external appearance were unaffected by triclosan treatment.

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

4.2	**Teratogenic / embryo toxic effects**	Foetal development showed retarded ossification at the high dose (cranium, vertebrae, sternebrae, metacarpals, and pelvic girdle, see Table A6_8_1-2). There were no other remarkable findings.
4.3	**Other effects**	–

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	This study was conducted to assess the potential for maternal and/or developmental toxicity of triclosan in the pregnant CD rat. Dosing suspensions and vehicle (1% carboxymethylcellulose in a 20% aqueous glycerol solution) were provided by the sponsor and administered by gastric intubation to mated female rats during the Day 6-15 gestation interval. Dose levels evaluated were 0 (vehicle control) 15, 50 and 150 mg/kg/day. Groups I and II were comprised of 24 mated animals and Groups III and IV were comprised of 25 mated animals. Animals were observed twice daily for mortality/morbidity and for obvious pharmacologic and/or toxicologic effects and each female was given a detailed physical examination on Days 0, 6-15 (daily) and 20 of gestation. Females were weighed on Days 0, 6, 8, 10, 12, 14, 16, 18 and 20 of gestation and food consumption data were recorded for Days 0-6, 6-11, 11-16 and 16-20 of gestation. All surviving females were sacrificed on Day 20 of gestation and given a gross postmortem evaluation. At necropsy, liver weights were recorded and the uteri were removed, weighed intact and evaluated for the number of live, dead and resorbed foetuses and implantation sites. The ovaries were also dissected free and the number of corpora lutea recorded. Foetuses recovered at this time were evaluated for external malformations/ variations, sexed and weighed. Subsequently, one-half of the foetuses in each litter were processed for visceral evaluation (microdissection procedure) and the remaining foetuses were processed for staining of the ossified structures with Alizarin Red S and evaluated for skeletal malformations and/or ossification variations.
5.2	**Results and discussion**	No treatment-related mortality was seen. Pregnancy rates were comparable between the control group and each treatment group and 21 control litters, 22 Group II (15 mg/kg/day) litters, 22 Group III (50 mg/kg/day) litters and 22 Group IV (150 mg/kg/day) litters were recovered at Day 20 gestation providing 303, 353, 346 and 349 foetuses, respectively, for evaluation in each of these groups. No maternal or developmental toxicity was seen at the 15 or 50 mg/kg/ day dose levels. At the 150 mg/kg/day dose level, a slight, although statistically significant decrease in food consumption was seen over the Day 6-11 gestation interval. This was the only suggestion of a maternal toxic effect. The only developmental toxicity seen at this high-dose level was retarded ossification as indicated from increases in the foetal and litter incidences of several ossification variations involving the cranium, vertebrae, sternebrae, metacarpals and pelvic girdle.
5.3	**Conclusion**	Triclosan was not a teratogenic agent under the conditions of this study. This conclusion is read across to DCPP.
5.3.1	LO(A)EL maternal toxic effects	LOEL = 150 mg triclosan/kg bw/day ≅ 132 mg DCPP/kg bw/day, based on decreased maternal food consumption
5.3.2	NO(A)EL maternal toxic effects	NOEL = 50 mg triclosan/kg bw/day ≅ 44 mg DCPP/kg bw/day

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

5.3.3	LO(A)EL embryo toxic / teratogenic effects	LOAEL = 150 mg triclosan/kg bw/day ≅ 132 mg DCPP/kg bw/day, based on retarded ossification
5.3.4	NO(A)EL embryo toxic / teratogenic effects	NOAEL = 50 mg triclosan/kg bw/day ≅ 44 mg DCPP/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2008
Materials and Methods	Applicant's version is adopted
Results and discussion	*4.1.4* Pregnancy rates, *litter size, fetal loss, litter and mean pup wights, preimplantation loss, sex distribution and number of resorptions were unaffected by triclosan treatment at doses of 15, 50 and 150 mg/kg/day. Furthermore*, body weight development, liver weights, and external appearance were unaffected by triclosan treatment.
Conclusion	*Maternal NO(A)EL 50 mg/kg bw/day based on reduced food intake and foetal NO(A)EL 50 mg/kg bw/day* *Triclosan was not teratogenic in the rat under the conditions of this study. External, visceral and skeletal evaluations of foetuses recovered from females treated with triclosan at dose levels of 15, 50 and 150 mg/kg/day indicated no increase in malformation rate. Triclosan was foetotoxic at 150 mg/kg bw/day seen as delayed ossification at several skeletal foci.*
Reliability	2
Acceptability	Acceptable
Remarks	*2.3 + 3.3.1* Treatment was terminated after gestation day 15. Although the OECD guideline 414 recommends an exposure through the entire period of gestation, this discrepancy is evaluated to be insignificant for the teratogenicities parameters, however reduced effects on foetotoxicity cannot be ruled out.

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1 Teratogenicity test in the rat

Table A6_8_1-1. Table for teratogenic effects (separate data for all dosage groups)

Maternal effects

Parameter	0 mg/kg	15 mg/kg	50 mg/kg	150 mg/kg	Dose-response + / –
Number of dams examined	24	24	25	25	
Mean food consumption (Day 6-11) [g/rat/day]	76	73	72	70*	+
*$p \leq 0.05$, Dunnett's test					

Table A6_8_1-2 Table for teratogenic effects (separate data for all dosage groups)

Examination of the foetuses

Parameter	0 mg/kg	15 mg/kg	50 mg/kg	150 mg/kg	Dose-response + / –
Incomplete ossification Foetal incidence [%]					
Metacarpals	0.7	4.1	3.0	8.3	+
Supraoccipital	15.0	25.6	23.1	31.4	+
Parietal	3.4	5.2	3.0	8.3	+
Malar	3.4	7.0	4.1	12.4	+
3rd Stenebrum	0.7	3.5	1.8	4.7	+
Ischium	1.4	2.9	2.4	6.5	+
Pubis	0.7	9.3	4.1	8.3	+

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1992b): A Segment II Teratology Study in Rabbits with [redacted] Project No. [redacted] date: 1992-04-16 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Company with letter of access	–	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No guideline statement, but general compliance with OECD Guideline 414 in its original (1981) version	
2.2	**GLP**	Yes	
2.3	**Deviations**	Deviations from current version of OECD 414: – Treatment was terminated after gestation day 18. – Relative humidity in the animal facility exceeded the recommended range (recommended by OECD 414: 50-60%, actual: 33-90%).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	**Triclosan**	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Not reported, specification is on file at the sponsor's facility	
3.1.2.1	Stability	Content, stability and homogeneity in vehicle was verified analytically	
3.2	**Test Animals**		
3.2.1	Species	Rabbit	
3.2.2	Strain	New Zealand White	
3.2.3	Source	[redacted]	
3.2.4	Sex	♀	
3.2.5	Age/weight at study initiation	Age: ~ 5 months Weight: 3028 - 4190 g	
3.2.6	Number of animals per group	18	
3.2.7	Control animals	Yes	
3.2.8	Mating period	Repeated 1-2-h mating periods with two males	
3.3	**Administration/ Exposure**	**Oral**	
3.3.1	Duration of exposure	Day 6-18 post mating	
3.3.2	Postexposure period	Day 19 -30 post mating	
3.3.3	Type	Gavage	

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

3.3.4	Dose levels	15, 50, 150 mg/kg bw/day
3.3.5	Vehicle	1% (w/w) carboxymethylcellulose in 20% aqueous glycerol
3.3.6	Concentration in vehicle	0.395%, 1.31%, 3.92% (w/w)
3.3.7	Total volume applied	3.8 mL/kg bw
3.3.8	Controls	Vehicle
3.4	**Examinations**	
3.4.1	Body weight	Days 0, 6, 8, 10, 12, 14, 16, 19, 24, and 30 of gestation.
3.4.2	Food consumption	Days of gestation: 1, 3, 5-19 (daily), 24 and 29
3.4.3	Clinical signs	Twice daily
3.4.4	Examination of uterine content	Number of implantations, number of corpora lutea, uterus weight, number of live and death foetuses, early and late resorptions
3.4.5	Maternal organ weights	Liver
3.4.6	Examination of foetuses	
3.4.6.1	General	Each foetus was individually identified, weighed, sexed externally (ano-genital distance) and given a gross examination for external mal-formations / variations to include observation for palatal defects. Late resorptions were weighed and discarded.
3.4.6.2	Soft tissue	All foetuses were evaluated for visceral malformations/ variations using a micro-dissection procedure. Evaluations were performed on the fresh foetal specimens shortly after removal from the uterus.
3.4.6.3	Skeleton	After the visceral inspection, the foetuses were eviscerated (internally sexed by inspection of the gonads) and processed for staining of the ossified skeletal structures using the Alizarin Red S staining procedure.
3.4.7	Statistics	Interval data: Statistical evaluation of equality of means was made by the appropriate ore way analysis of variance technique, followed by a multiple comparison procedure if needed. First Bartlett's test was performed to determine if groups had equal variance. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one way ANOVA using the F distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated a summed rank test (Dunn) was used to determine which treatments differed from control. A statistical test for trend in the dose levels was also performed. In the parametric case (i.e., equal variance) standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's Statistic for monotonic trend was used. The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1% two-sided risk level. All ratios were transformed via Bartlett's transformation followed by the

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

arc sine transformation prior to analysis. Data are presented untransformed.

Incidence data: Statistical analysis of incidence data was performed using contingency tables. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. Next, each treatment group was compared to the control group using a 2x2 Fisher Exact Test; the significance level was corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Thirdly, Armitage's test for linear trend in the dosage groups was performed. In keeping with standard statistical practice, if any one cell had an expected value less than 5, the chi-square and Armitage's tests were not reported. When this occurred, only the Fisher Exact test (corrected via Bonferroni inequality) was performed and reported.

3.5 Further remarks –

4 RESULTS AND DISCUSSION

4.1 Maternal toxic effects

4.1.1 Clinical signs: No effects

4.1.2 Mortality: There was no treatment-related mortality.

4.1.3 Body weight: At 150 mg/kg/day: significant decrease in body weights and bw gain during gestation (see Figure A6_8_1-1).

4.1.4 Food consumption: At 150 mg/kg/day: significant decrease in food consumption during both treatment and post-treatment interval (see Figure A6_8_1-2).

4.1.5 Other: Pregnancy rates, liver weights, and necropsy findings were unaffected by triclosan treatment.

4.2 Teratogenic / embryo toxic effects Embryonic and foetal development was unaffected by treatment.

4.3 Other effects –

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

This study was conducted to assess the potential for maternal and/or developmental toxicity of triclosan in the pregnant New Zealand White rabbits. Dosing suspensions and vehicle (1% carboxymethylcellulose in a 20% aqueous glycerol solution) were provided by the sponsor and administered by gastric intubation to mated female rats during the Day 6-18 gestation interval. Dose levels evaluated were 0 (vehicle control) 15, 50 and 150 mg/kg/day. Each group was comprised of at least 18 mated does.

Animals were observed twice daily for mortality/morbidity and for obvious pharmacologic and/or toxicologic effects and each female was given a detailed physical examination on Days 0, 6-15 (daily), 20 and 30 of gestation. Females were weighed on Days 0, 6, 8, 10, 12, 14, 16, 19, 24 and 30 of gestation and food consumption data were recorded for Days 1, 3, 5 through 19 (daily), 24 and 29 of gestation. All surviving females were sacrificed on Day 30 of gestation and given a gross postmortem evaluation. At necropsy, liver weights were recorded and the uteri were removed, weighed intact and evaluated for the number of live, dead and resorbed foetuses and implantation sites. The ovaries were also dissected free and the number of corpora lutea recorded. Foetuses recovered at this

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

		time were evaluated for external malformations/ variations, sexed and weighed. Subsequently, all foetuses were processed for visceral evaluation (microdissection procedure) and then processed for staining of the ossified structures with Alizarin Red S and evaluated for skeletal malformations and/or ossification variations.
5.2	**Results and discussion**	No adverse effect of treatment with triclosan was evident from mortality or pregnancy rates. At the Day 30 gestation sacrifice, 16 control litters, 15 Group II (15 mg/kg/day) litters, 15 Group III (50 mg/kg/day) litters and 16 Group IV (150 mg/kg/day) litters were recovered providing 143 Group I, 126 Group II, 129 Group III and 124 Group IV foetuses for evaluation. At dose levels of 15 and 50 mg/kg/day no maternal or developmental toxicity was evident. At the 150 mg/kg/day level, maternal toxicity was evident from the following responses; lower gestation weights during the treatment period; reduced weight gain over the entire Day 6-19 gestation interval; and reduced food consumption over the treatment period. No developmental toxicity was indicated at the 150 mg/kg/day dose level. Triclosan was not teratogenic in the rabbit under the conditions of this study. External, visceral and skeletal evaluation of foetuses recovered from females treated with this test substance at dose levels of 15, 50 and 150 mg/kg/day indicated no increase in malformation rate.
5.3	**Conclusion**	Triclosan was not a teratogenic agent under the conditions of this study. This conclusion is read across to DCPP.
5.3.1	LO(A)EL maternal toxic effects	LOEL = 150 mg triclosan/kg bw/day ≅ 132 mg DCPP/kg bw/day, based on decreased bw gain
5.3.2	NO(A)EL maternal toxic effects	NOEL = 50 mg triclosan/kg bw/day ≅ 44 mg DCPP/kg bw/day
5.3.3	LO(A)EL embryo toxic / teratogenic effects	LOAEL > 150 mg triclosan/kg bw/day ≅ 132 mg DCPP/kg bw/day, no developmental effects
5.3.4	NO(A)EL embryo toxic / teratogenic effects	NOAEL = 150 mg triclosan/kg bw/day ≅ 132 mg DCPP/kg bw/day
5.3.5	Reliability	1
5.3.6	Deficiencies	No

Section A6.8.1 Teratogenicity Study

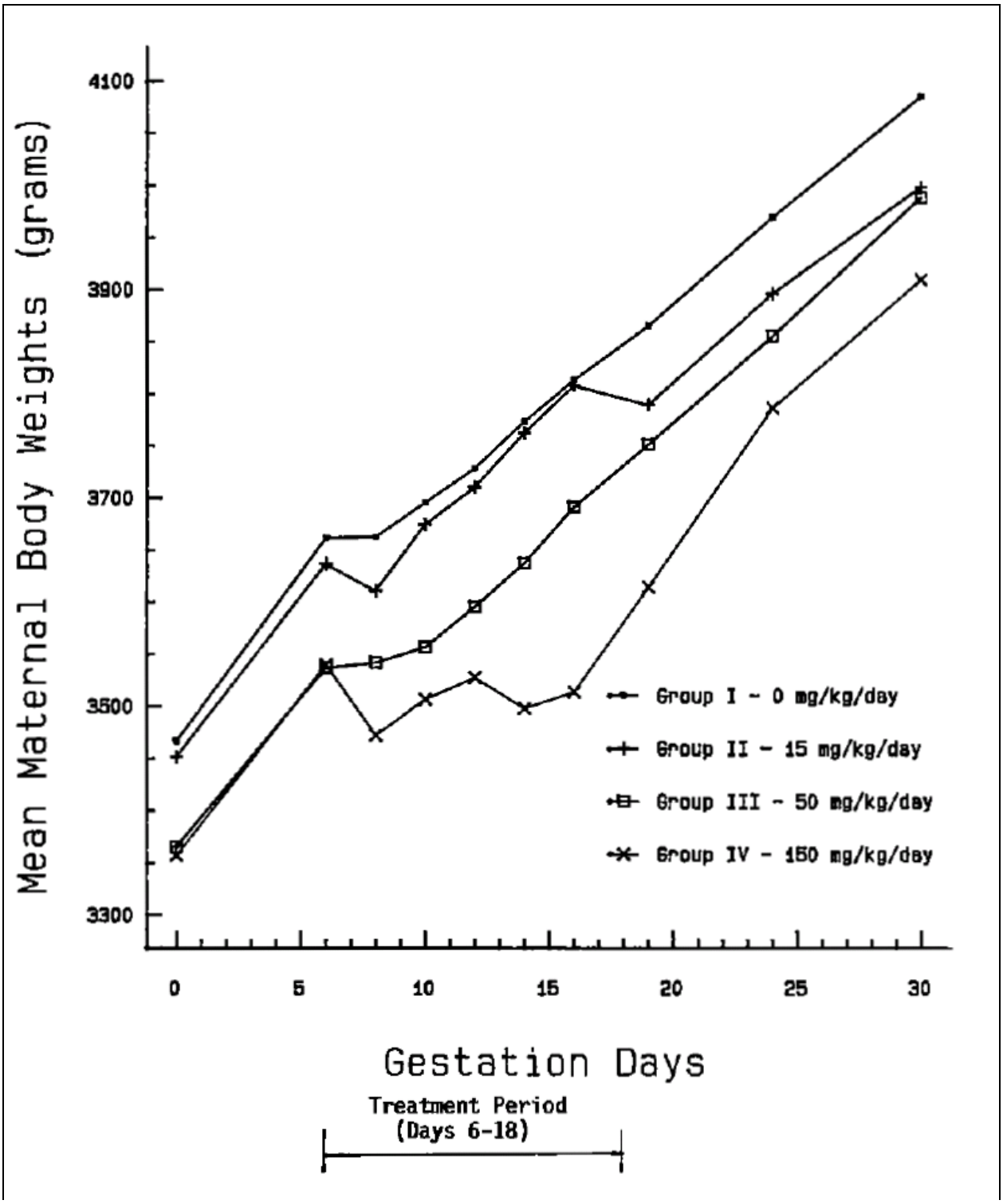
Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2008
Materials and Methods	*2.3* Deviations from current version of OECD 414: – Treatment was terminated after gestation day 18. – Relative humidity in the animal facility exceeded the recommended range (recommended by OECD 414: 50-60%, actual: 33-90%). – *Only 18 instead of 20 animals per group were used.* *4.1.5* Pregnancy rates, *litter size, fetal loss, litter and mean pup wights, preimplantation loss, sex distribution and number of resorptions were unaffected by triclosan treatment at doses of 15, 50 and 150 mg/kg/day. Furthermore* liver weights and necropsy findings were unaffected by triclosan treatment.
Results and discussion	Applicant's version is adopted
Conclusion	Applicant's version is adopted
Reliability	2
Acceptability	Acceptable
Remarks	*2.3* Treatment was terminated after gestation day 18. The OECD guideline 414 recommends an exposure through the entire period of gestation, this discrepancy is evaluated to be insignificant for the teratogenicities parameters. However, reduced effect on foetotoxicity cannot be ruled out.

Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

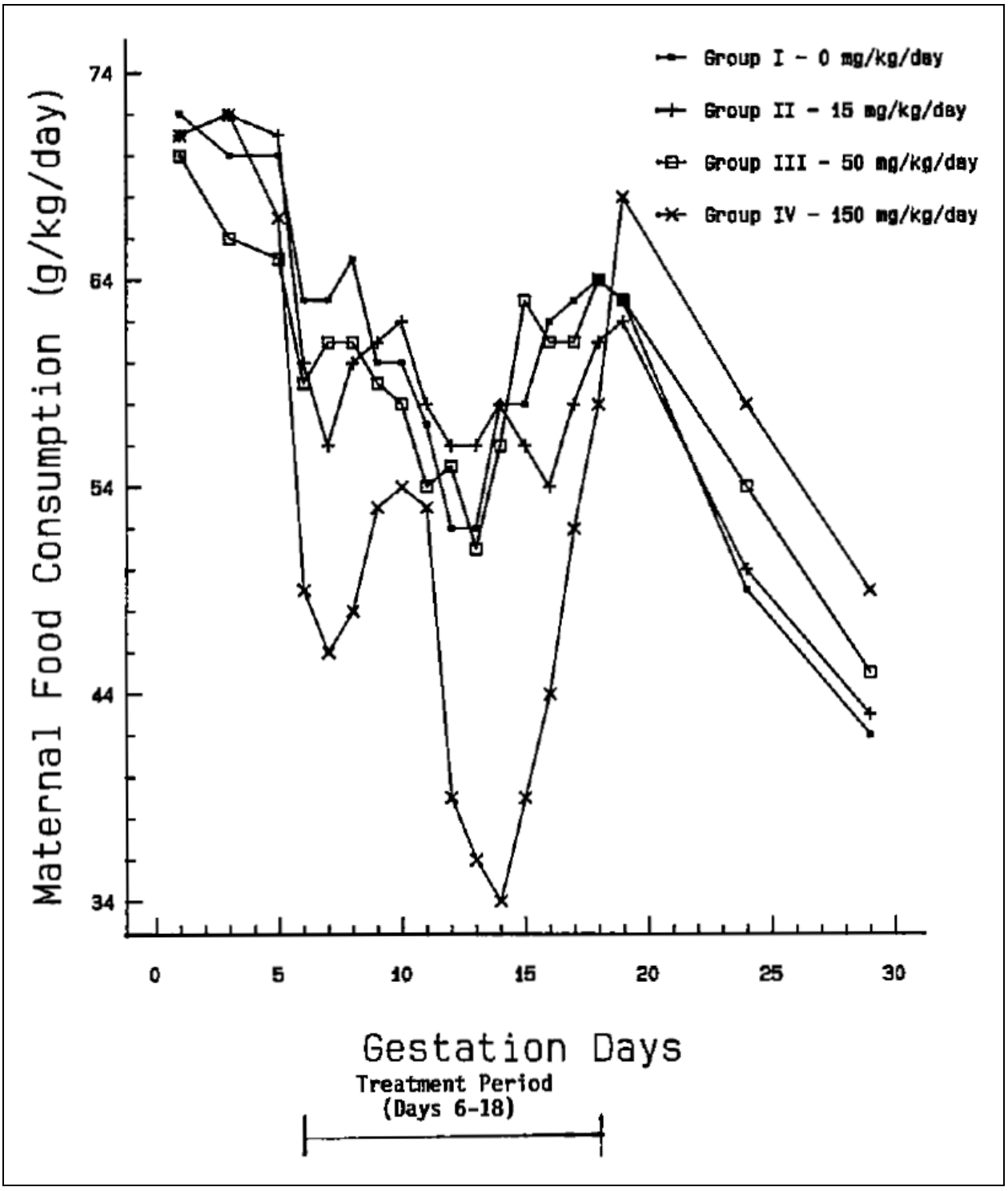
Figure A6_8_1-1. Mean maternal body weights during gestation



Section A6.8.1 Teratogenicity Study

Annex Point IIA VI.6.8.1 6.8.1(2) Teratogenicity test in the rabbit

Figure A6_8_1-2 Mean maternal food consumption during gestation



Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](1988): Two-Generation Reproduction Study in Rats – [redacted] Study No.[redacted] date: 1988-03-18 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Company with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes US-EPA FIFRA Guideline 83-4 (≅ OECD Guideline 416)
2.2	**GLP**	Yes
2.3	**Deviations**	– The mating period was up to 31 days (recommended: two weeks). – The required organ weights were not determined. – Sperm parameters were not assessed. – No histopathology was performed.

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	
3.1.2.1	Description	White powder with lumps
3.1.2.2	Purity	≥ 99%
3.1.2.3	Stability	Stability and homogeneity in diet was analytically verified
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Sprague-Dawley (Crl:CD®(SD)Br)
3.2.3	Source	[redacted]
3.2.4	Sex	♂+♀
3.2.5	Age/weight at study initiation	6 weeks of age, ♂: 195.8-229.8 g bw, ♀: 147.1-172.3 g bw
3.2.6	Number of animals per group	25/sex/group (F_0) 30/sex/group (F_1)
3.2.7	Mating	1:1
3.2.8	Duration of mating	Up to 31 days
3.2.9	Deviations from standard protocol	Litters were culled to 8 pups/litter on lactation Day 4.
3.2.10	Control animals	Yes

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

3.3	**Administration/ Exposure**	Oral
3.3.1	Animal assignment to dosage groups	See Table A6_8_2-1 below.
3.3.2	Duration of exposure before mating	F0 parents: 10 weeks; F1 parents: 10 weeks
3.3.3	Duration of exposure in general P, F1, F2 males, females	F0 parents: from study initiation until scheduled sacrifice F1 parents: from weaning until scheduled sacrifice F2 pups: during lactation via milk
		Oral
3.3.4	Type	in food
3.3.5	Concentration	300, 1000, 3000 ppm
3.3.6	Actual doses	Excel sheet with the underlying calculations is attached. F0 ♂: 22.8, 76.8, 237.8 mg/kg/day (pre-mating) F0 ♀: 27.6, 91.3, 284.5 mg/kg/day (pre-mating) 22.6, 64.5, 232.7 mg/kg/day (gestation) 49.3, 151.9, 481.5 mg/kg/day (lactation) F1 ♂: 31.4, 104.3, 342.6 mg/kg/day (pre-mating) F1 ♀: 33.1, 111.8, 359.4 mg/kg/day (pre-mating) 21.7, 75.6, 242.1 mg/kg/day (gestation) 30.5, 119.0, 310.6 mg/kg/day (lactation)
3.3.7	Vehicle	Diet
3.3.8	Controls	Plain diet
3.4	**Examinations**	
3.4.1	Mortality	Twice daily
3.4.2	Clinical signs	Once daily
3.4.3	Body weight	Weekly during treatment and at terminal sacrifice for ♂ and ♀ that were not confirmed mated. Confirmed mated ♀ were weighed on presumed gestation Days 0, 7, 14, and 20. Nursing dams were weighed on Days 0, 4, 7, 14, and 21 post partum.
3.4.4	Food consumption	Weekly during pre-mating in ♂ and ♀. On Days 7, 14, and 20 of gestation and on Days 4, 7, 14, and 21 of lactation. No food consumption data was obtained during the mating period.
3.4.5	Oestrus cycle	Daily during mating.
3.4.6	Sperm parameters	No
3.4.7	Offspring	Sex-ratio, pup viability, body weight gain, clinical signs, gross necropsy on dead pups
3.4.8	Organ weights P and F1	Not determined
3.4.9	Histopathology P and F1	Not performed

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

3.4.10	Histopathology F1 not selected for mating, F2	Not performed
3.5	**Further remarks**	The test substance intake was not reported but has been calculated by the author of this dossier. The underlying calculations are provided as an Excel spreadsheet. A suggestion of an effect on the kidneys (dilated renal pelvis) was seen in the F1 weanling necropsy data (Groups 3 and 4) but was not evident in the F1 adult sacrifice or the F2 pup necropsy data.
		4 RESULTS AND DISCUSSION
4.1	**Effects**	
4.1.1	F0 (♂+♀)	No effects
4.1.2	F1 (♂+♀)	3000 ppm: reduced viability index (survival for Days 0-4) reduced bw during lactation in F1 pups reduced bw during gestation in F1 dams see Table A6_8_2-1
4.1.3	F2 (♂+♀)	No effects
4.2	**Other**	–
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	This study was designed to evaluate the reproductive toxicity of triclosan when fed daily to male and female rats for two generations To this end parental (F0, F1) mating behaviour and fertility as well as gestation parturition and lactation were investigated in addition to offspring (F growth and development. The test material was mixed with diet at concentrations of 300, 1000, or 3000 ppm (Groups 2-4, respectively) The control group (Group 1) received the basal diet. Twenty-five rats per sex per group were assigned to the study. Breeding was initiated after a ten-week growth phase. Thirty F1 rats per sex per group were selected for breeding to produce the F2 generation The growth phase for the F1 animals ranged from approximately 12-14 weeks. All F2 pups were sacrificed following weaning. Body weight and food consumption data were collected at selected intervals throughout the study. Reproductive and developmental toxicity was assessed by measuring duration of gestation, pregnancy rates, fertility, and pup viability growth, and development.
5.2	**Results and discussion**	There was no evidence of treatment related deaths in the parental generation. Clinical and cageside observations of the F0 and F1 animals during growth, gestation, and lactation did not indicate a compound-related effect. During the F0 growth phase the mean body weight values occasionally were below control levels in Group 4; however, the mean gain values did not indicate a treatment-related effect. The F0 female mean body weight and food consumption values during gestation also did not indicate a treatment-related effect. During lactation the mean Group 4 mean body weight values were less than the corresponding control values, however, the mean weight gains were similar to the control gains. Therefore, no clear evidence of toxicity was indicated in clinical observation data, mean body weight values and food consumption values for the F0 animals during any phase.

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

In F0 females, there was no indication of treatment influencing oestrous cycling, pregnancy rate, and gestation length. The gestation index was 100% for all groups.

The F1 viability index (survival for Days 0-4) was similar for Groups 1-3, but substantially lower for Group 4 pups. The weaning index (survival for Days 4-21), however, was similar for all groups.

An apparent high-dose effect in the F1 pups was evidenced by a significant reduction in the mean adjusted body weights of both sexes during lactation. The F1 mean body weights during the growth phase were below control values in both Groups 2 and 4, but generally similar to control values In Group 3. The mean body weight change values for the growth phase were generally similar each week, but a significant negative trend was displayed for the females for total mean changes (Weeks 0-11). This pattern also was evident for the males but not statistically significant. In both cases, Group 3 values were similar to the control values. Again, no evidence of a treatment-related effect was seen in the clinical observation and mean food consumption data.

Mean F1 body weight values (Day 0, 7, 14, 20) and body weight change values (0-7, 14-20, 0-20) during gestation were significantly below the control values in Group 4. This was also seen in Groups 2 and 3 but only for the Day 20 weight. Group 4 mean lactation body weight values were also below the control levels, but the mean weight gain values did not indicate a treatment-related effect.

A suggestion of an effect on the kidneys (dilated renal pelvis) was seen in the F1 weanling necropsy data (Groups 3 and 4) but was not evident in the F1 adult sacrifice or the F2 pup necropsy data.

F1 pregnancy rates did not indicate a dose-related effect. No influence of treatment on normal cycling behaviour of the F1 females was indicated. Length of gestation was similar for all groups. Survival of the F2 pups to weaning was slightly lower in Group 4 compared to the control group. Although Group 4 mean pup weight values were significantly below the control value at birth, they were above or similar to the control values at all other times.

In conclusion, triclosan at these dietary levels did not cause any adverse effects on reproductive performance of the adults. Evidence of neonatal toxicity at the highest dose level (3000 ppm) was indicated in the survival of the F1 pups and suggested for the F2 pups. No indication of adverse effects on the pups at the lower two dose levels was suggested.

Evidence of toxicity was seen in the F1 pup mean body weight data at the highest dose level during lactation this was not evident for the F2 pups. Though no dose-related evidence of toxicity in the growth phase of either generation was noted, the mean body weight values for both the high- and low-dose groups of the F generation were lower than the control values while the values of the mid-dose group were similar to the control values.

Based on these data, the highest no adverse effect level is probably 1000 ppm.

5.3 Conclusion

5.3.1	LO(A)EL	
5.3.1.1	Parent males	LOAEL > 3000 ppm, no adverse effects ≅ 210 mg DCPP/kg/day (pre-mating intake by F0 ♂)
5.3.1.2	Parent females	LOAEL > 3000 ppm, no adverse effects

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

		≅ 251 mg DCPP /kg/day (pre-mating intake by F0 ♀)
5.3.1.3	F1 males	LOAEL = 3000 ppm, based on reduced viability index ≅ 424 mg DCPP/kg/day (F0 maternal intake during lactation)
5.3.1.4	F1 females	LOAEL = 3000 ppm, based on reduced maternal bw during gestation ≅ 213 mg DCPP /kg/day (F1 maternal intake during gestation)
5.3.1.5	F2 males	LOAEL > 3000 ppm, no adverse effects ≅ 274 mg DCPP /kg/day (F1 maternal intake during lactation)
5.3.1.6	F2 females	LOAEL > 3000 ppm, no adverse effects ≅ 274 mg DCPP /kg/day (F1 maternal intake during lactation)
5.3.2	NO(A)EL	
5.3.2.1	Parent males	NOAEL = 3000 ppm ≅ 210 mg DCPP /kg/day (pre-mating intake by F0 ♂)
5.3.2.2	Parent females	NOAEL = 3000 ppm ≅ 251 mg DCPP /kg/day (pre-mating intake by F0 ♀)
5.3.2.3	F1 males	NOAEL = 1000 ppm ≅ 134 mg DCPP /kg/day (F0 maternal intake during lactation)
5.3.2.4	F1 females	NOAEL = 1000 ppm ≅ 67 mg DCPP /kg/day (F1 maternal intake during gestation)
5.3.2.5	F2 males	NOAEL = 3000 ppm ≅ 274 mg DCPP /kg/day (F1 maternal intake during lactation)
5.3.2.6	F2 females	NOAEL = 3000 ppm ≅ 274 mg DCPP /kg/day (F1 maternal intake during lactation)
5.3.3	Reliability	2
5.3.4	Deficiencies	Organ weights and histopathology parameters were not assessed. However, the main purpose of a two-generation reproduction study is to identify effects on reproductive parameters and this purpose is fully met by the present study. Organ weights are determined in other repeated-dose studies and histopathology of reproductive organs is also common practice in these studies. No remarkable findings were made in the various repeated-dose studies. Sperm parameters were also not assessed, but the fact that reproductive performance was not impaired in the present study allows the conclusion that the number and motility of spermatozoa was not reduced by the test compound. The present study is therefore valid for assessment of potential hazards to reproduction by triclosan and DCPP.

Section A6.8.2 Multigeneration Reproduction Toxicity Study

Annex Point IIA VI.6.8.2 6.8.2 Oral two-generation study in the rat

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date **EVALUATION BY RAPPORTEUR MEMBER STATE**

Materials and Methods 20 May 2008

Results and discussion *2.3*: The mating period was up to 31 days (recommended: two weeks).

- The required organ weights were not determined.
- Sperm parameters were not assessed.
- No histopathology was performed.
- *No functional investigations (e.g. motor activity, sensory function, reflex ontogeny) were included.*

Conclusion Applicant's version is adopted

Reliability *5.3.4* Organ weights and histopathology parameters were not assessed. The main purpose of a two-generation reproduction study is to identify effects on reproductive parameters and this purpose is *not* fully met by the present study. Organ weights are determined in other repeated-dose studies and histopathology of reproductive organs is also common practice in these studies. Sperm parameters were also not assessed in this study and the fact that t*he mating period was prolonged can hide negative effects on sperm quality.*

The prolonged mating period indicates a possible negative effect on male reproductive performance that should be further investigated. It is therefore not possible to state that the reproductive performance was not impaired in the present study due to the prolonged period of mating.

The present study is only valid for assessment of potential hazards to female reproduction but not to male reproduction by triclosan.

Acceptability 2

Remarks -

Table A6_8_2-1. Table for reproductive toxicity study

Parameter		Genera-tion	Controls		300 ppm		1000 ppm		3000 ppm		Dose-response	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Body weight	g											
average weight on gestation Day 14		**F_0 dams**	/	348.4	/	338.6	/	356.0	/	343.8	/	–
		F_1 dams	/	357.1	/	339.1	/	344.7	/	316.7*	/	+
average pup weight on lactation Day 21		**F_1 pups**	44.8	42.8	44.6	41.8	46.3	43.5	40.2*	37.7*	+	+
		F_2 pups	40.7	39.5	41.0	39.3	44.1	41.2	38.5	36.9	–	–
Reproductive Performance												
Viability index	%	**F_1 pups**	90		94		96		82*		+	
		F_2 pups	87		97		90		84		–	

* statistically significant different from control $p \leq 0.05$

Section 6.9 **Annex Point IIIA VI. 1**	**Neurotoxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	No special investigations in animals have been conducted to evaluate the neurotoxicity potential of DCPP. DCPP does not possess structural similarity to known neurotoxicants such as organophosphates or carbamates. General toxicity studies did not yield indications for a specific neurotoxic potential of DCPP.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the applicant´s version.	
Conclusion	Agree with the applicant´s version.	
Remarks	-	

Section A6.12 Human Case Report

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

Official use only

1 REFERENCE

1.1	**Reference**	[redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	–
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA.

2 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

3 MATERIALS AND METHODS

3.1	**Substance**	[redacted]Triclosan
3.2	**Persons exposed**	[redacted]

4 RESULTS

4.1	**Results of examinations**	[redacted]- regularly receive medical check-ups according to national requirements (Gefahrstoffverordnung, Biostoffverordnung), to local regulations of accident insurance (Berufsgenossenschaft der chemischen Industrie) as well as to Environment, Health and Safety Policy of [redacted] Health surveillance of employees of triclosan production by an occupational health professional is guaranteed. Workplace health risk assessments are performed regularly. In the context of medical check-ups over a decade of time medical complaints of production employees, which may be attributed to Irgasan DP 300, were not observed. In particular did no diseases occur, which required declaration to local accident insurance as potential occupational disease. Ambient monitoring is part of measures of occupational hygiene surveillance of employees of Triclosan production.
4.2	**Conclusion**	No problems related to handling / production of triclosan were reported.

Section A6.12 Human Case Report

Annex Point IIA VI.6.9.1 6.12.1 Medical surveillance data on manufacturing plant personnel

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2012
Materials and Methods	Agree with the Applicant´s version.
Results and discussion	Agree with the Applicant´s version.
Conclusion	Agree with the Applicant´s version.
Remarks	-

Section 6.12.2 Annex Point IIA VI.6.9.2	Direct observation, e.g. clinical cases, poisoning incidents if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Reports on clinical cases related to DCPP exposure were not found in the published literature. The oral toxicity of DCPP is very low and poisoning incidents are very unlikely.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.3 Annex Point IIA VI.6.9.3	Health records, both from industry and any other available sources	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Medicinal surveillance of personnel involved in the production and formulation of DCPP did not reveal adverse health effects. The literature search did not produce findings on the contrary.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.4 Annex Point IIA VI.6.9.4	Epidemiological studies on the general population, if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No adverse health effects associated with DCPP exposure have been reported in the medical literature.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*Give date of action*	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.5 Annex Point IIA VI.6.9.5	Diagnosis of poisoning including specific signs of poisoning and clinical tests, if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	DCPP is non-toxic via all relevant routes of exposure. In all likelihood, no specific signs of poisoning are going to occur. Clinical tests are not available.	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*October 2012*	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.6 Annex Point IIA VI.6.9.6	Sensitisation/allergenicity observations, if available	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	No observations on sensitisation by or allergy towards DCPP were available in the literature.	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.7 Annex Point IIA VI.6.9.7	Specific treatment in case of an accident or poisoning: first aid measures, antidotes and medical treatment, if known	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	DCPP is non-toxic via all relevant routes of exposure. Accidental ingestion or poisoning with DCPP has never been reported. Specific first aid measures, antidotes and medical treatment have therefore never been developed.	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.12.8 Annex Point IIA VI.6.9.8	Prognosis following poisoning	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	DCPP is non-toxic via all relevant routes of exposure. Poisoning with DCPP has never been reported. The prognosis after accidental ingestion of DCPP is good.	
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Agree with the Applicant´s version.	
Conclusion	Agree with the Applicant´s version.	
Remarks	-	

Section 6.13 Annex Point IIIA VI. 2	Toxic effects on livestock and pets	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	There is practically no potential for exposure of livestock or pets to DCPP from its proposed use in product types 1, 2, and 4. Food bowls for pets may be cleaned with DCPP-containing dishwashing fluid. The residues taken up by a pet are going to be on the same order of magnitude as the secondary exposure of humans (about 0.3 pg DCPP/kg bw/day). This exposure is too low to be of toxicological relevance. Pets may ingest spills from DCPP-containing cleaning liquid (PT 2). This is an acute exposure scenario. Judging from the acute toxicity data for experimental animals, DCPP is essentially non-toxic via the oral route in rats and via the dermal route in rats and rabbits. Long-term studies in rodents demonstrated that no relevant effects are to be expected upon chronic oral exposure to DCPP. Thus, any long-term exposure of pets or livestock to low doses of DCPP is deemed harmless.	x
Undertaking of intended data submission [ ]		
Evaluation by Competent Authorities		
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	PT1: No exposure of pets is considered. PT2: Dermal and oral exposures of pets (e.g. cats and dogs) are assumed to reveal a comparable pattern and situation in comparison to the secondary exposure scenario derived for infants (low bodyweight, direct oral uptake from floor and transfer from skin to mouth). Therefore, exposure of pets is also assumed to be low and in the same order of magnitude PT4: Oral exposures of pets (e.g. cats and dogs) are possible, if bowls intended for pets (food, water) are washed with DCPP-containing dishwashing detergents. This situation is considered to be comparable to the secondary exposure scenario determining potential oral uptake of humans. As low exposure levels were identified in the latter case, exposure of pets is also assumed to be low (not relevant).	
Conclusion	Please see text above.	
Remarks	-	

Section 6.15.1 **Section 6.15.2** **Annex Point IIIA XI.1**	**Identification / behaviour of the residues (identity and concentrations), degradation and reaction products and of metabolites of the active substance in contaminated foods or feedingstuffs**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [...] **Limited exposure [X]**	**Technically not feasible [X]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The estimation of exposure via diet from residual DCPP on dishes has revealed very low exposure from this source, 28 ng/kg bw/day. This is equivalent to 1.6 µg/day for a 60 kg consumer. If a daily ration of 1 kg is consumed, this intake would be equivalent to a concentration of 1.6 µg/kg food or 1.6 ppb. The development of an analytical method to reliably determine the concentration of DCPP or its potential degradation products in food is technically not feasible for this order of magnitude. It is not expected that food contamination with DCPP will be a significant source of human exposure.	x
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	October 2012	
Evaluation of applicant's justification	Assuming oral uptake of DCPP-residues via washed dishes (complying with 0.42 mg treatment solution per day), a concentration of 0.0004% of the treatment solution and an oral absorption of 100%, results in a systemic exposure level of 1.68x10-6 mg DCPP/d via the dermal route respectively 2.80x10-8 mg/kg bw/d referring to a bodyweight of 60 kg (default, adult). 0.42 mg/d x 0.000004 x 1 /60 kg = $2.80x10^{-8}$ mg/kg bw/d	
Conclusion	An analytical method in food matrix (vegetable oil) has been provided (cf. Doc. II-A)	
Remarks	-	

Section 6.15.3 Estimation of potential or actual exposure of the active substance to humans through diet and other means

Annex Point IIIA XI.1.4

Official use only

1 REFERENCE

1.1 Reference [redacted] (2007): BPD Dossier on DCPP. Doc. II-B (PT 4), Section 3.1.4

1.2 Data protection No

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study No applicable guideline

3 MATERIALS AND METHODS

3.1 Model The exposure to DCPP from its use in dishwashing concentrates was estimated using the respective default scenario of ConsExpo 4.1.

The in-use concentration of DCPP in the suds is 0.004%.

ConsExpo assumes that 0.42 mg of the dishwashing solution remains on dishes and can be ingested each day. A quality factor of 2 was assigned to this figure. This means that the parameter is based on single data source supplemented with personal judgement.

The body weight of the consumer is assumed to be 60 kg.

4 RESULTS AND DISCUSSION

4.1 Results The daily exposure to DCPP from its proposed use in dishwashing concentrates is 2.8×10^{-7} mg/kg bw/day = 28 ng/kg bw/day.

The proposed ADI for DCPP is 0.66 mg/kg bw/day. The anticipated exposure via diet maintains a margin of exposure to this ADI of 2.5×10^{6}.

The potential overall exposure to DCPP from its use in dishwashing concentrates is 6.48×10^{-4} mg/kg bw/day. The exposure via diet makes up only 0.04% of the total exposure predicted for the proposed use of DCPP.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Conclusion The estimation demonstrates that exposure via diet to DCPP does not make a significant contribution to the potential overall exposure to DCPP.

5.1.1 Reliability 0

This is an estimation based on a mathematical model for which standard criteria for assigning reliability factors do not apply.

5.1.2 Deficiencies No

Section 6.15.3 Estimation of potential or actual exposure of the active substance to humans through diet and other means

Annex Point IIIA XI.1.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2012
Materials and Methods	n.a.
Results and discussion	See conclusion given below
Conclusion	This document supports the applicant's exposure assessment. In the course of detailed evaluation content of the exposure assessment was amended by the competent authority. The acceptable intended use is as given in Doc. II-B chapter 3. The accepted human exposure assessment is as given in Doc. II-B chapter 4.
Reliability	n.a.
Acceptability	Acceptable with the amendments/replacements given above
Remarks	n.a.

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

Summary from applicant

SUMMARY

In vitro and in vivo studies with Triclosan of unknown quality revealed a number of adverse effects that could not be reproduced with USP grade material in studies according to internationally accepted guidelines. Studies that intentionally depressed T4 levels in dams and offspring revealed learning deficits and other developmental neurotoxicity in rats which were preceded by effects on the gonads, the thyroid and body weight development. The latter effects were absent in studies with Triclosan at doses that would be expected to lead to a similar T4 depression. In addition, based on ADME studies that revealed large differences between rats and humans, a study in rats may have little relevance to humans. Moreover, because it has been shown that Triclosan leads to T4 depletion by receptor-mediated upregulation of hepatic catabolism, it should first be shown that this adverse outcome pathway has any relevance to humans.

RELEVANCE OF PUBLISHED STUDIES

While it is true that some studies in the public domain point to an estrogenic, as well as anti-estrogenic, anti-androgenic and androgenic activity of Trilcosan in cell lines and reporter assays, the quality of the test material has often not been considered.

In vivo results, such as the study by Kumar et al. [1] who reported effects on androgen regulation with concomitant effects on testis including weight and histopathological malformations. These effects were accompanied by reduced sperm density in the epididymis at doses as low as 20 mg/kg bw/day. There is no reference to the analytical purity of the test material other than a percentage purity of the Indian material used. It remains unclear whether this grade of Triclosan meets the USP (United States Pharmacopeia) specification which specifically restricts polychlorinated dibenzo-p-dioxins and -furans.

In contrast to Kumar's findings, several studies in the rat conducted with USP grade material found no effect on the morphology of the testes or epididymis with doses up to ca. 300 mg/kg bw (90-day treatment, covering at least one spermatogenic cycle [2]) or up to ca. 150 mg/kg bw (two year treatment [3]). In addition, no effects on the testes or epididymis were observed in a two generation study [4] with this test material which suggests that USP grade Triclosan does not cause these effects in rats. Studies with USP grade Triclosan in other species such as the mouse, beagle dogs or baboons also found no effect on the gonads [5-9]. The incidence of regressed testes in the hamster lifetime bioassay is thought to be secondary to a treatment related decrement in body weight gain [10]. This is species-specific to the hamster as a seasonal breeder that has evolved certain mechanisms for the spontaneous regression of testicular tissue at times of the year when breeding is not possible.

Conflicting results were obtained for effects on female reproductive organs. The study by Stoker et al. [11] is as an example for the estrogenic effects of Triclosan in vivo, which resulted in advanced vaginal opening and altered estradiol levels. This is contrasted by a study published in the same year [12] that found delayed vaginal opening at similar doses of Triclosan in addition to other reproductive effects (lowered sex ratio, lowered pup body weights).

EFFECTS ON THE TYHROID AXIS

The registrant is engaged in a CRADA (cooperative research and development agreement) with the US EPA that investigates the effect of USP quality Triclosan on thyroid hormone homeostasis. The following publications document the available results: a recent study by Zorilla et al. of the US EPA found a depression of T4 levels in male weanling rats upon exposure to Triclosan which had no effect on the onset of puberty [13]. A second study performed by the US EPA in female weanling rats was designed to elucidate the mechanism of action of Triclosan on thyroid hormone homeostasis [14]. The Paul et al. study confirmed the effect on T4 found in male rats and found evidence for upregulation of hepatic catabolism with the initiating event most likely being the activation of hepatic CAR

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

and PXR receptors. Hepatic catabolism, in turn, is thought to increase T4 conjugation by way of phase II glucoronidation and sulfation which leads to the observed T4 depletion. Because enzyme induction studies in rodents often fail to predict enzyme induction in humans [15], it is presently unclear if this mechanism has any relevance to the human situation.

There are several studies in the public domain that studied downstream effects of T4 depression. Some of the studies tested industrial chemicals for their developmental neurotoxicity; others studied the effects of an imbalance in thyroid hormone homeostasis by deliberately knocking down T4. Although the primary effect of T4 depression is common to all studies including the above mentioned studies conducted by the US EPA with Triclosan, the downstream consequences differ. Also, the combined results from these studies suggest that certain parameters appear to be key elements in the assessment of developmental neurotoxicity. Three studies were recently published by the National Food Institute of the Technical University of Denmark. Axelstad et al. conducted developmental neurotoxicty studies with Mancozeb (a fungicide) [16] and the UV-filter octyl methoxycinnamate [17] and found no behavioural effects. In the former study, a dose dependent decrease in T4 in dams was observed down to 63% of controls at the high dose level while the T4 levels in the offspring were unaffected. No effects on reproductive organs or behavioural changes were observed. The latter study, too, found no typical behavioural abnormalities and no effects on preputial separation or anogenital distance, however, a delayed testes development and lower prostate weight (compared to controls) with accompanying histological changes were observed. T4 levels were as low as 4% and 0% of controls in dams of the mid and high dose group while in pups, T4 was depressed to 64% of controls in both the mid and high dose group. The third study explored the effect of propylthiouracil (PTU) in rats and found effects on weight and histology of the thyroid glands as well as neurodevelopmental defects in offspring with T4 levels of 23% of controls and below [18]. In the Zorilla et al. study [13], T4 was in the same range, namely as low as 20% of controls, with no effect on androgen-dependent tissue weights, testosterone levels, T3, TSH or the onset of preputial separation. A study recently presented at the annual meeting of the American society of toxicology that was conducted as a range finder to a dermal carcinogenicity study found no effect on sperm morphology, motility, cauda sperm counts, testes sperm counts or the estrous cycle stage (determined by vaginal cytology measurements) [19]. In this study, T4 was knocked down to less than 50% of the control values.

Additional studies found developmental neurotoxicity that was accompanied by decreased litter size, increased pup mortality, decreased pup survival, slower body weight development [20]; increase in thyroid weight in the offspring with concomitant histological abnormality of the follicular epithelium, testis and body weight depression, increase in relative brain and thyroid weight in females [21]; and body weight gain depression in the offspring even after T4 values had returned to normal [22]. Especially the retarded development of the pups appears to be a consistent pattern in T4 depressed rats. In addition, histopathological changes and body/ organ weight effects appear to precede effects in behavioural tests which became evident in work performed by Axelstad et al. [16, 17] and Noda [21]. In the study by Noda et al., for instance, hearing disturbances and an increase number of errors in the Biel-type and T-maze tests were observed in the high dose group which were accompanied by morphological changes in the thyroid. These changes were still evident in the mid dose group where no neurotoxic effects were observed. This is consistent with the registrant's experience that morphological changes are more sensitive than behavioural tests. Numerous studies with repeated exposure in six species with doses up to 900 mg Triclosan/kg bw/day failed to show effects on the gonads or the thyroid gland (especially absence of hyperplasia and morphological changes) and the two generation study in rats revealed no effects on gonad development, litter size, pup viability and survival, thyroid weight and histology as well as body weight gain development. Delayed body weight development, which is also a relevant endpoint in man suffering from hypothyroidism, should have been evident even after T4 levels had returned to normal in the two generation study which was due to the low exposure of the pups to Triclosan from

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

maternal milk. Hence, in the absence of hints in studies with Triclosan on adverse effects triggered by T4 depression such as the aforementioned, it seems unlikely that this imbalance triggers any downstream effects.

VALIDITY OF THE TWO GENERATION STUDY

The two generation study available with USP1 grade Triclosan was conducted to a previous version of OECD guideline 416 that did not require the determination of sperm parameters [4]. This suggests that subtle changes in these parameters may have been missed due to the study design, especially because it was conducted with the hyperfertile rat (p. 10, para 4, draft decision). An evaluation by Mangelsdorf et al. (BAuA) found a strong correlation between histopathology data, organ weights and male fertility. The authors concluded that these data from repeated dose studies may be used to identify adverse effects on male reproduction [23]. Histopathology and organ weights taken from 90 day studies were in fact shown to be more sensitive than fertility parameters that were measured during multi-generation studies. It could also be shown that exposure for 4 weeks suffices for an assessment of male fertility, although 90 day studies have been regarded as superior in the past because they cover a complete cycle of spermatogenesis [23]. If such a 28 day (or 90 day) study reveals neither significantly elevated testis or ovary weights nor histopathological alterations in those organs, the weight of the evidence is that effects on reproduction are also not expected [24]. A comparison of more than one hundred 90 day studies with two-generation studies that used the same test substance additionally showed that the NOAELs differed by less than the variation limit of studies, i.e. a factor of two [25]. Therefore, the information gained from a two-generation study can be regarded as minimal if a 90 day study has been performed.

Effects on the weight and histopathology of the gonads of several species are absent in studies conducted with USP grade Triclsoan. In conclusion, although the present OECD guideline 416 requires fertility parameters that were not assessed during the two-generation study available, the available data are sufficient to assess the endpoint ‘male fertility’.

RELEVANCE OF THE RAT

Oral absorption of Triclosan is almost complete in all species tested (mice, rats, hamsters, guinea pigs, rabbits, dogs and monkeys). Pharmacokinetic and distribution data suggest that enterohepatic circulation occurs in the rat and mouse but appears to be absent in humans and hamsters.

All species tested so far exhibit complete metabolization of the parent compound to the glucuronide and sulfate conjugate. In a number of species, including the rat, the sulfate conjugate dominates the glucuronide with the feces being the primary rout of excretion. In hamsters and humans the glucuronide conjugate predominates in urine which is the primary route of excretion of these two species. The elimination half-lives in hamsters and humans are additionally similar (but different from those in the rat and mouse). Depending on the conditions, a metabolic shift towards the sulfate conjugate may occur in both hamsters and humans, while a complete re-conjugation to the glucuronide occurs before excretion in urine which is unique to these two species. The fact that oral absorption, metabolism and excretion are comparable in hamsters and humans suggests that the hamster is the most relevant species for human risk assessment. A recent study that compared all available animal data based on a benchmark-dose approach, confirmed that the hamster, of all animals tested thus far, is the most sensitive species [26].

As stated above, the induction of phase II catabolism in rats may have no relevance to humans as it is receptor-mediated [15], which makes it necessary to clarify whether this initiating event of the adverse outcome pathway has any relevance to the human situation.

REFERENCES

1. Kumar, V., et al., Alteration of testicular steroidogenesis and histopathology of reproductive system in male rats treated with triclosan. Reprod Toxicol, 2009. 27(2): p.

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

177-85.

2. Ciba (2001), [redacted] 13-week oral toxicity (gavage) study in the Wistar rat, report number RCC project number [redacted] 30 Mar 2001

3. Ciba-Geigy (1986), [redacted] 2-year oral administration to rats, report number [redacted] 28 Apr 1986

4. Ciba-Geigy (1988), Two-generation reproduction study in rats [redacted] report number HLA study No. [redacted] 18 Mar 1988

5. Ciba-Geigy (1993), Subchronic oral toxicity study of Triclosan in CD-1 mice, report number [redacted] 28 Jan 1993

6. Ciba-Geigy (1976), Eighteen-month carcinogenicity study with [redacted] in albino mice, report number [redacted] 25 May 1976

7. Ciba-Geigy (1983), 90-day oral toxicity study in rats with [redacted] final report, report number [redacted] Oct 1983

8. Ciba-Geigy (1970), 90 days oral toxicity study in Beagle dogs with [redacted] 10 Jul 1970

9. Ciba-Geigy (1975), 1 year oral toxicity study in Baboons with compound [redacted], report number [redacted] 28 Jul 1975

10. Ciba-Geigy (1999), [redacted] Potential tumorigenic and chronic toxicity effects in prolonged dietary administration to hamsters, report number [redacted] 30 Mar 1999

11. Stoker, T.E., E.K. Gibson, and L.M. Zorrilla, Triclosan exposure modulates estrogen-dependent responses in the female wistar rat. Toxicol Sci, 2010. 117(1): p. 45-53.

12. Rodriguez, P.E. and M.S. Sanchez, Maternal exposure to triclosan impairs thyroid homeostasis and female pubertal development in Wistar rat offspring. J Toxicol Environ Health A, 2010. 73(24): p. 1678-88.

13. Zorrilla, L.M., et al., The effects of triclosan on puberty and thyroid hormones in male Wistar rats. Toxicol Sci, 2009. 107(1): p. 56-64.

14. Paul, K.B., et al., Short-term exposure to triclosan decreases thyroxine in vivo via upregulation of hepatic catabolism in Young Long-Evans rats. Toxicol Sci, 2010. 113(2): p. 367-79.

15. Cassarett, L.J.I., Cassarett & Doull's Toxicology: the basic science of poisons. 7th ed., ed. C.D. Klaassen2007, New York, N.Y. (USA).

16. Axelstad, M., et al., Exposure to the widely used fungicide mancozeb causes thyroid hormone disruption in rat dams but no behavioral effects in the offspring. Toxicol Sci, 2011. 120(2): p. 439-46.

17. Axelstad, M., et al., Effects of pre- and postnatal exposure to the UV-filter octyl methoxycinnamate (OMC) on the reproductive, auditory and neurological development of rat offspring. Toxicol Appl Pharmacol, 2011. 250(3): p. 278-90.

18. Axelstad, M., et al., Developmental neurotoxicity of propylthiouracil (PTU) in rats: relationship between transient hypothyroxinemia during development and long-lasting behavioural and functional changes. Toxicol Appl Pharmacol, 2008. 232(1): p. 1-13.

19. Jia-Long Fang, M.V., Frederick A. Beland, Beth E. Juliar, Greg Olson, Ralph Patton, Mason G. Pearce, Wafa Harouk, 13-week dermal toxicity of Triclosan in B6C3F1 mice, 2013, National Center for Toxicological Research, Jefferson, Arkansas 72079: American Society of Toxicology Annual Meeting 2013.

20. Brosvic, G.M., J.N. Taylor, and R.E. Dihoff, Influences of early thyroid hormone manipulations: Delays in pup motor and exploratory behavior are evident in adult operant performance. Physiology & Behavior, 2002. 75(5): p. 697-715.

21. Noda, S., et al., Preliminary evaluation of an in utero-lactation assay using 6-n-

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

propyl-2-thiouracil. Archives of Toxicology, 2005. 79(7): p. 414-421.

22. Provost, T.L., et al., Dose- and age-dependent alterations in choline acetyltransferase (ChAt) activity, learning and memory, and thyroid hormones in 15- and 30-day old rats exposed to 1.25 or 12.5 ppm polychlorinated biphenyl (PCB) beginning at conception. Progress in Neuro-Psychopharmacology & Biological Psychiatry, 1999. 23(5): p. 915-928.

23. Mangelsdorf, I., J. Buschmann, and B. Orthen, Some aspects relating to the evaluation of the effects of chemicals on male fertility. Regul Toxicol Pharmacol, 2003. 37(3): p. 356-69.

24. Mangelsdorf, I.B., J (2002), Extrapolation from results of animal studies to humans for the endpoint male fertility, report number

25. Janer, G., et al., A retrospective analysis of the two-generation study: what is the added value of the second generation? Reprod Toxicol, 2007. 24(1): p. 97-102.

26. Rodricks, J.V., et al., Triclosan: a critical review of the experimental data and development of margins of safety for consumer products. Crit Rev Toxicol, 2010. 40(5): p. 422-84.

Evaluation by Competent Authorities

Date 2013-11

Summary from RMS

The data submitted by the applicant are not sufficient to conclude on the endocrine disrupting properties of triclosan or DCPP, as no histopatological or biochemical investigation of the influence on thyroidea effects have been carried out. There are, however, a growing number of studies from the open literature supporting that triclosan is a potential endocrine disrupter.

In frogs triclosan has been described to have weak estrogenic activity (Ishibashi et al., 2004), weakly androgenic activity in fish (Foran et al., 2000) and it was reported that triclosan alters thyroid hormone-associated gene expression and the rate of thyroid hormone-mediated postembryonic anuran development (Veldhoen et al., 2006).

Several studies have reported changes of thyroid homeostasis in rats after triclosan treatment. Crofton et al. (2007), Zorilla et al. (2009) & Paul et al. (2010) reported decreases in T4 levels and minor decreases in T3, with unaffected TSH levels in weanling male rats. Zorilla et al. (2009) and Paul et al. (2010) also carried out mechanistic studies in order to elucidate the underlying mode of action. In microsomes and tissue samples from triclosan treated rats both studies found increased PROD1 activity (a marker for CYP2B induction) and decreased EROD2 activity (a marker for CYP1A1 induction, AhR mediated). These findings are further supported by increased CYP2B mRNA expression and decreased CYP1A mRNA expression as reported by Paul et al. (2010). They further found increased CYP3A as well as UGT and SULT expression on the mRNA level. Paul et al. (2010) suggested that the mode of action of triclosan-induced decrease in thyroxin in rats may be partially due to up regulation of hepatic catabolism. These findings are largely supported by the studies by Studie A6.10(01), Study A.10(02) and Study A6.10(03) where enzyme induction was observed in the livers of rats, mice and hamsters upon triclosan treatment (see section 3.7.3 as well as Section 3.12, p.**Fehler! Textmarke nicht definiert.**).

Screening the similarities of triclosan using the OECD QSARs toolbox indicates that the substances is a strong binder of the estrogen receptor. Steroids and steroid mimics have been shown to activate PXR &/or CAR receptors, important regulators of steroid and xenobiotic detoxification enzymes and transporters (phases I-III). Among other downstream consequences the activation of PXR &/or CAR results in CYP2B and CYP3A induction, thereby increasing steroid and thyroid hormone metabolism

[1]PROD: Pentoxyresorufin-O-deethylase, a marker for CYP2B induction.

[2]EROD: Ethoxyresorufin-O-deethylase, a marker for CYP1A1 induction. Often induced by Ah receptor activation.

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

(Kretschmer & Baldwin, 2005). Using in vitro reporter assays it could be demonstrated that triclosan activates the human PXR (Jacobs et al., 2005).

Recently anti-androgenic effects (i.e. suppression of steroidogenesis, ↓ testis weight, ↓ weight of accessory sex tissues) were described in the rat (Kumar et al., 2009), however, this study had several deficiencies: among others impurities were not defined and the reported reduction of testis weight in this study was not seen in repeated dose toxicity studies, with high purity triclosan (see section 3.5). On the other hand delayed mating success observed in the 2-generation study by Study A 6.8.2 would support possible testicular effects (see section 3.8).

It should further be noted that in two hamster studies testicular regression as well as reduced numbers of germ cells and spermatozoa were reported. In the sub-chronic Study A 6.4.1/02 these effects were seen in all groups including the control males. However, in the chronic hamster study by Study A 6.5, 6.7/02 testicular regression and reduced numbers of spermatozoa and germ cells as well as atypical spermatozoa reached significance only in the high dose group. The applicant concluded that these effects were caused by the fact that the affected hamsters were in the supressed phase of the breeding cycle, as hamsters are seasonal breeders. While this might be plausible for the sub-chronic study, where all males were affected including those of the control group, it is not argueable that in the chronic study only high dosed males were in the supressed phase of the breeding cycle. It is also not plausible that seasonal regression of male reproductive organs leads to abnormal spermatogenic cells.

Seasonal gonadal regression as well as profound reductions in food intake and body weight was described to be linked to reduction of thyroid hormones (mainly T3) caused by decreasing day length in Siberian hamsters (Barret et al., 2007). Similar effects were also described for other mammalian and bird species (e.g. Parkinson and Follett, 1994, Yoshimura et al. 2003). Effects on the male reproductive organs observed in the chronic hamster study might therefore represent supporting evidence for the influence of triclosan on thyroid homeostasis. Triclosan has been shown to elicit reproductive toxicity in adult rat and fetal toxicity in offspring exposed in utero, during lactation and after weaning (Rodriguez and Sanchez, 2010). At all doses tested a significant decrease in sex-ratio of the pubs was observed (less male pubs) along with significantly reduced pub weight at postnatal day 20 and significantly delayed vaginal opening. Further, the results demonstrated a dose-related decrease in the live birth index and in the 6-day survival index reaching significance in the high dose group (50 mg/kg/day). Weights of uteri and ovaries were not affected. A dose dependent decrease in T4 and to a lesser extent in T3 levels in the blood of the dams was seen during pregnancy and lactation. Vaginal opening has not been investigated in the rat 2-generation study by Study A 6.8.2, but decreased foetal body weight and decreased day 0-4 survival were only reported at much higher doses (260 mg/kg/bw).

In a study by Stoker et al. (2010), triclosan was found to affect oestrogen-mediated responses in the pubertal and in the weanling female rat and also to suppress thyroid hormone in both studies. Triclosan did not increase uterine weight alone, but together with estradiol treatment at concentrations ≥ 4.7mg/kg/bw. Delayed vaginal opening and increased uterine weight was seen at 150 mg/kg/bw. T4 levels were reduced at concentrations ≥19mg/kg/bw, with a NOEL of 9.4 mg/kg/bw.

Overall these studies showed equivocal results with regard to estrogenic and anti-androgenic activity of triclosan, whereas the observed effects on thyroid hormone homeostasis were rather consistent between the different studies.

It is generally accepted that thyroid tumours in the rat are of minor relevance to humans due to the high sensitivity of the rat thyroid-pituitary-hypothalamic (HPT) axis. However, it is not possible to also draw such a general conclusion on other effects on the thyroid axis– especially as thyroid effects have been reported to be increasing within the human population (Boas et al., 2006). Disturbance of the HPT axis could in principle be considered of relevance for humans since regulation of thyroid function is rather similar in rats and humans, despite the higher susceptibility of rats.

In a review on thyroid-disrupting chemicals Miller et al. (2009) summarised several epidemiological studies and concluded that even small, transient, decreases in serum total T4 are associated with altered brain development. Low maternal serum thyroxin during gestation has previously been demonstrated to correlate with four- to 10-point IQ deficit in children (Haddow et al. 1999).

There is only a limited body of literature on endocrine related human health effects of triclosan exposure. However, six studies have been summarised below to address the possible endocrine

Section 6.15.5 **Any other available information that is relevant (Annex Point IIIA XI.1)**

Potential endocrine disruption

	disruptive effect of triclosan. Effects on thyroid hormones in adults using toothpaste containing triclosan was investigated in a study by Allmyr et al. (Allmyr et al., 2009). During the 14 days study, a significant increase in plasmaconcentrations was found among the 12 adults. However, no relationship with thyroid hormones was found. In two studies investigating 1239 and 90 girls, respectively, pubic hair development together with breast development as well as effect on BMI was investigated in pre-pubertal girls via single spot urine samples (Wolff et al., 2010 and Wolff et al., 2007). A statistically significant inverse correlation between pubic hair development and triclosan was found, but no correlation with breast development or BMI could be established. Another two studies examined the correlation between prenatal triclosan exposure and birth weight via a single spot analysis in 404 and 48 mother-newborn pairs, respectively, but no associations was found other than a statistically insignificant association between triclosan and birth length by Wolff et al. (Wolff et al., 2008 and Phillipat et al., 2012). A further prenatal study examined the association between triclosan and undescended testes as well as hypospadias in 48 mother-newborn pairs. No association was found, however, in the case of hypospadias the result might have been influenced by the small sample size (Chevrier et al., 2012). The studies are mainly based on small sample sizes and spot sample analysis. Therefore, no final conclusions can be drawn from these studies regarding the thyroid disrupting potential of triclosan in humans and they cannot be used to rule out a possible endocrine disruptive effect. Among other effects reduction of thyroid hormone levels has been described to be induced by dioxins (Boas et al., 2006), which are common impurities of triclosan. The test material investigated by Paul et al. (2010) contained 0.12% 2,8-dichlorodibenzodioxin and 0,1% 2,4,8-trichlorodibenzodioxin. It was discussed whether the impurities contained in the test material could be responsible for the effects observed. Paul et al. (2010) reported that they did not observe activation of EROD2, which is observed after Ah-receptor activation, induced by dioxins. However, a competitive interaction between triclosan and TCDD activated Ah-receptor activation has also been discussed. Though it is shown that the dioxins might have an effect on thyroid hormone levels, it is assumed that it will only contribute insignificantly to the overall endocrine disruptive effect of triclosan. Based on the above, triclosan must be considered to be a suspected endocrine disrupting compound. A scientific and regulatory review of triclosan is currently being conducted by the U.S. Food and Drug Administration (FDA) in collaboration with the U.S. Environmental Protection Agency (EPA). FDA is reviewing FDA-regulated products and EPA is specifically studying the potential endocrine disrupting effects of triclosan. Publication of final results is expected in winter 2012. Triclosan will be subject to further evaluation under REACH where it has been included on the Community Roling Action Plan (CoRAP) and will undergo substance evaluation (REACH Article 44-48). The substance evaluation will start in 2012 and is targeted to two identified areas of concern: PBT and endocrine disruption. The substance evaluation process is limited to one year after which a substance evaluation report will be published. Based on this development it is appropriate to postpone the assessment of this issue until the evaluation under REACH has been finalised.
Conclusion	It is appropriate to postpone the assessment of this issue until evaluation criteria for ED are agreed in Europe and the evaluation under REACH has been finalised
Remarks	-

Section A7.1.1.1.1 Annex Point IIA, VII 7.6.2.1 Hydrolysis as a function of pH and identification of breakdown products

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](1999g): Hydrolysis determination of [redacted] at different pH values [redacted], unpublished, Date: 1999-03-01.
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD guideline 111 EEC Directive 92/69, C.7
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	Non-radiolabelled test substance DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Non-radiolabelled test substance
3.1.3	Purity	> 99%
3.1.4	Further relevant properties	Water solubility of DCPP = 19.5 mg/L at 20 °C; OECD 105 (Tognocci, 712012)
3.2	**Reference substance**	None
3.2.1	Initial concentration of reference substance	-

Section A7.1.1.1.1 Annex Point IIA, VII 7.6.2.1 — Hydrolysis as a function of pH and identification of breakdown products

3.3	**Test solution**	The study was carried out with buffer solutions at three pH levels: - pH 4: 1.64 g sodium acetate were dissolved in 200 mL water from Milli Q supply. 6 g acetic acid were filled up to 1000 mL with water. 166 mL of the sodium acetate solution and 834 mL of the acetic acid solution were combined to get 1000 mL buffer solution pH 4.0. - pH 7: 3.41 g imidazol were dissolved in 250 mL water from Milli Q supply. 254 mL 0.1 m hydrochloric acid and 250 mL imidazol solution were filled up to 1000 mL with water. - pH 9: Borate/Potassium chloride/NaOH (Baker Art. No. 7145) The buffer solutions were sterilized for 25 minutes in an autoclave prior to first use. Nitrogen was passed through the buffer solutions for 5 minutes except when freshly sterilized. All buffers solutions were described in Table A7_1_1_1_1-1.
3.4	**Testing procedure**	- Preliminary test -
3.4.1	Test system	The test was performed according to the OECD guideline 111 and EEC Directive 92/69, C.7. The test item was dissolved in the buffer solutions and incubated at a specified temperature with the water bath kept constant at ± 0.1 °C, usually. The concentration of the test item was determined as a function of time at each pH. pH 4.0: 10.21 mg of DCPP were dissolved in 100 mL buffer solution (pH 4.0) containing 2% dimethylsuplhoxide as solubilizer. pH 7.0: 10.83 mg of DCPP were dissolved in 100 mL buffer solution (pH 7.0) containing 2% dimethylsupihoxlde as solubilizer. pH 9.0: 10.11 mg of DCPP were dissolved in 100 mL buffer solution (pH 9.0) containing 2% acetonitrile as solubilizer. The mixtures were ultrasonified and submitted to a 0.45 µm filtration. To obtain a test solution of not more than half the water solubility, the solutions were diluted 1:1 with the respective buffer.
3.4.2	Temperature	50 °C
3.4.3	pH	4 / 7 / 9
3.4.4	Duration of the test	5 days
3.4.5	Number of replicates	Two aliquots of each test solution of approximately 50 mL each were transferred into 50 mL Erlenmeyer flasks in order to perform a duplicate test.
3.4.6	Sampling	The sampling intervals were: 2.4 and 120 hours (5 days).
3.4.7	Analytical methods	Determination of residual DCPP residues by high performance liquid chromatography (HPLC) using standard solutions.

Section A7.1.1.1.1 Annex Point IIA, VII 7.6.2.1 — Hydrolysis as a function of pH and identification of breakdown products

Standard solutions:
10.66 mg of DCPP were dissolved in 100 mL acetonitrile/water (60:40; v/v) to prepare a stock solution of 106.6 µg/mL DCPP. This stock solution was diluted with the same solvent mixture to obtain six standard solutions in the range between 2.132 µg/mL and 79.95 µg/mL DCPP.

Chromatographic conditions:

Column:	Hypersil ODS, part. Size 5 µm, 250 x 4 mm		
Mobile phase:	Eluent A: Water (+ 2 g TBAHS per L)		
	Eluent B: Acetonitrile (+ 2 g TBAHS per L)		
	0 min.	60%A	40%B gradient
	0-10 min.	0% A	100% B gradient
	10-11 min.	0% A	100% B gradient
Equilibrium time:	10 min.		
Detection:	232 nm		
Flow rate:	1.0 mL/min.		
Temperature:	room temperature		
Sample size:	50 µL		

Apparatus:

Workstation:	Varian Workstation LC 9020 STAR
Pump:	Varian Pump 9010
Autosampler:	Varian Autosampler 9300
Detector:	Varian Detector 9050

Retention time:

DCPP:	about 6.9 min

3.5 Preliminary test — Yes

4 RESULTS

4.1 Concentration and hydrolysis values — At 50 °C: <10% degradation after 5 days at pH 4, 7 and 9.

See Table A7_1_1_1_1-2

4.2 Hydrolysis rate constant (k_h) — Not reported

4.3 Dissipation time — DT_{50} (s) at 50 °C:

Stable

DT_{50} (s) at 25 °C:

pH 4: > 365 days
pH 7: > 365 days
pH 9: > 365 days

4.4 Concentration – time data — The initial and final concentration of the test substance in the different buffer solutions and different sampling points are reported in Table A7_1_1_1_1-2.

4.5 Specification of the transformation products — Since no degradation has been observed transformation products do not have to be specified

Section A7.1.1.1.1
Annex Point IIA, VII 7.6.2.1

Hydrolysis as a function of pH and identification of breakdown products

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The hydrolytic stability of DCPP was tested according to the OECD guideline 111 and EEC Directive 92/69, C.7 at pH levels of 4, 7, and 9 at 50 °C in buffer solutions for the preliminary test. The test duration was 2.4 hours and 5 days.
5.2	**Results and discussion**	DCPP showed no significant degradation at pH 4, 7 and 9 at 50 °C. It has a half-life period longer than one year at 25 °C at pH 4, 7 and 9. Therefore, DCPP was considered to be hydrolytically stable. Formation of hydrolysis products was not observed in the course of the study. Considering the high hydrolytic stability determined under stringent temperature conditions and at different pH values it is not expected that hydrolytic processes will contribute to the degradation of DCPP in aquatic systems.
5.2.1	k_h	A value for k_h is not given in the report; since no degradation is observable, this is not meaningful
5.2.2	DT_{50}	A value for DT_{50} is not given in the report; since no degradation is observable, this is not meaningful
5.2.3	r^2	A value for r^2 is not given in the report; since no degradation is observable, this is not meaningful
5.3	**Conclusion**	Validity criteria can be considered as fulfilled.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2010
Materials and Methods	-
Results and discussion	4.3:Clarification DT_{50} (s) at 50 °C (preliminary test): Stable; less than 10% degradation after 5 days at pH 4, 7 and 9. Conclusion: DT_{50} (s) at 25 °C: pH 4: > 365 days pH 7: > 365 days pH 9: > 365 days
Conclusion	5.2: Clarification DCPP showed no significant hydrolytical degradation at pH 4, 7 and 9 at 50 °C (preliminary test). Therefore, a half-life period longer than one year at 25 °C at pH 4, 7 and 9 is concluded. DCPP is considered to be hydrolytically stable.
Reliability	1
Acceptability	Acceptable with the amendments above
Remarks	-

Table A7_1_1_1_1-1: Type and composition of buffer solutions

pH	Type of buffer
4	Sodium acetate buffer: 1.64 g sodium acetate (Fluka Art. No. 71180) + 200 mL water from MiIIi Q supply. 6 g acetic acid (99-100%) (Baker Art. No. 6001) + 1000 mL water. 166 mL sodium acetate solution + 834 mL acetic acid solution = 1000 mL buffer solution pH 4.0.
7	Imidazol buffer solution: 3.41 g imidazol (Aldrich Art. No. 120-2) + 250 mL water from Milli Q supply. 254 mL 0.1 m hydrochloric acid (Baker Art. No. 7038) + 250 mL imidazol solution were filled up to 1000 mL with water.
9	Borate/Potassium chloride/NaOH (Baker Art. No. 7145).

Table A7_1_1_1_1-2: Preliminary test: Hydrolysis of DCPP at pH 4, 7 and 9, respectively after different incubation times (50 °C)

	Preliminary test at 50 °C					
	pH 4		**pH 7**		**pH 9**	
Run	1	2	1	2	1	2
Initial measured conc. [µg/mL]	2.700	2.804	1.085	1.025	32.003	32.064
Conc. measured after 2.4 hours incubation [µg/mL]	2.729	2.667	1.063	1.048	32.386	32.586
Conc. measured after 5 days incubation [µg/mL]	2.931	2.915	1.195	1.157	32.829	32.958
Degradation after 5 days incubation [%]	-	-	-	-	-	-

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 Phototransformation in water including identity of transformation products (01)

Official use only

1 REFERENCE

1.1 Reference

(1) [redacted] (2008): ^{14}C-DCPP Aqueous Photolysis Under Laboratory Conditions and Determination of the Quantum Yield.
[redacted]
Report No. [redacted] date: 2008-12-16, (unpublished).

Addendum:
(2) [redacted] (2009): Aqueous Photolysis of DCPP; Metabolite Identification by LC/MS.
[redacted]
Report No. [redacted] date: 2009-01-09, (unpublished).

1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	OECD Guideline for Testing of Chemicals, Proposal for a new Guideline: Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, Draft Document, August 2000. EPA-540/9-82-021, Section 161-2, October 18, 1982 EPA 540/09-90-078, December 1989 JMAFF Agchem Test Guidelines 12 Nohsan N. 8147, 24 November 2000, revised 26 June 2001: Photodegradation in Water (2-6-2)
2.2	**GLP**	Yes
2.3	**Deviations**	None

3 MATERIALS AND METHODS

3.1	**Test material**	^{14}C-DCPP
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Not reported
3.1.3	Purity	100 %
3.1.4	Radiolabelling	Yes
3.1.5	UV/VIS absorption spectra and absorbance value	The absorption spectrum of the test item in buffer solutions at pH 7 was obtained by measuring the absorbance with a spectrophotometer capable recording UV/VIS spectra between 200 nm and 800 nm. It could be shown that the test item absorbs light only below 400 nm.
3.1.6	Further relevant properties	-

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 — Phototransformation in water including identity of transformation products (01)

3.2 Reference substances

Non-labelled DCPP

3.3 Test solution

see table A7_1_1_1_2-1

3.4 Testing procedure

3.4.1 Test system

Photolysis was performed using cylindrical vessels constructed entirely of Pyrex glass and covered with quartz glass plates (to cut off radiation below 290 nm similarly to the natural sunlight cut-off by ozone), which were screwed on top of the vessel. The light was allowed to enter the solution only from the top of each vessel. For the collection of volatile compounds, the vessels were connected to an air flow-through system, with a gas flow sufficient to pass through the samples. The incoming air was passed through a water trap then through the vessels and finally through two traps to absorb any volatile compounds formed (e.g. $^{14}CO_2$). The traps were in the sequence 2 N NaOH and ethylene glycol and contained about 50 mL of each solution. All glass equipment (including the incubation vessels) was sterilized prior to use by rinsing with an ethanol/water (70:30; v/v) solution. The organic solvent was allowed to evaporate before the start of the study.

Duplicate samples of 100 mL test solution were transferred to sterilized test vessels (diameter about 6 cm, exposed area 28.26 cm^2) and continuously illuminated for 19 days (first application). A second application was made and duplicate samples of 100 mL test solution were treated in the same manner but illuminated for up to 6 hours (0.25 days), in order to additionally assess photolysis during the first hours of irradiation. The solutions were continuously stirred with magnetic stirrers.

In addition to the irradiated reaction vessels, a single sample containing 100 mL test solution was incubated under identical conditions but in the dark (control sample).

3.4.2 Properties of light source

The study was performed in a “Suntest CPS, Original Hanau” apparatus (Heraeus, Germany), equipped with a 1.8 kW xenon burner and an UV filter system.

Xenon Burner:	Max. 765 W/m^2 at max. UV filtering (lambda < 800 nm) with irradiance between 400 W/m^2 and 765 W/m^2 to a pre-set value.
Filters:	UV filter with a 290 nm cut-off to simulate natural sunlight.
Exposure Area:	Approximately 500 cm^2 total area
Vessel:	Exposed area 28.26 cm^2; 100 mL solution
Light Intensity:	44.1 W/m^2 in the 300 to 400 nm region of the spectrum was determined with a LI-1800 spectroradiometer (Li-Cor Inc./USA).
Optical path length:	3.5 cm

3.4.3 Determination of irradiance

For the determination of the number of photons entering the test solution and to calculate the quantum yield, the p-nitroanisole (PNA) actinometer was used. The PNA is one of the actinometers recommended by EPA and OECD.

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 — Phototransformation in water including identity of transformation products (01)

3.4.4	Temperature	The samples were maintained at a mean temperature of 24.8 ± 0.2 °C for the irradiated and 25.1 ± 0.4 °C for the dark control samples throughout the study.
3.4.5	pH	The experiment was carried out in buffer solutions at pH 7.
3.4.6	Duration of the test	The duration of the test was 19 days.
3.4.7	Number of replicates	2 replicates were incubated.
3.4.8	Sampling	Duplicate (irradiated) or individual (dark control) aliquots of 4 mL were taken for radioactivity measurement and analysis after the following days of irradiation/incubation: Irradiated: 0, 0.04, 0.08, 0.17, and 0.25 (second application) and 1, 2, 5, 9, and 19 days (first application) Dark control: 0, 1, 2, 5, 9 and 19 days After sampling, the pH of each sample was measured. Thereafter, the samples were directly submitted to LSC measurement and HPLC analysis. TLC was additionally conducted on selected samples. For metabolite identification in study (2) ^{12}C-DCPP was irradiated for 4, 8, and 12 hours and aliquots were subsequently analyzed by LC/MS.
3.4.9	Analytical methods	The radioactivity was determined by Packard liquid scintillation counters equipped with DPM and luminescence options (TRI-CARB 2500TR, 2700TR, 2750TR/LL or QuantaSmart 2900TR). HPLC was used to quantify the radioactive fractions present in the samples and to analyze the concentration of the actinometer solution. TLC analysis was used for selected samples to confirm the results obtained by HPLC. LC/MS analysis was used for the identification of metabolites. LC/MS analysis was used for metabolite identification.
3.4.10	Method of calculation	The radioactivity detected in the test solutions was expressed in % of the initial radioactivity applied. HPLC was routinely used to quantify the photodegradation products. The photolytic half-life (DT_{50}) and DT_{90} values were calculated by applying simple first-order reaction kinetics for the parent compound and consecutive first order reaction kinetics for the major metabolites M1, M7, and M8 using the least squares parameter estimation program Origin version 6.1 (Origin Lab Corporation, One Roundhouse Plaza, Northhampton, MA 01060 USA). The Suntest irradiation time was converted to natural midsummer sunlight time at latitudes from 30 - 40°N (Florida to New York) and 50°N (Central Europe) and natural spring sunlight time 35°N (Tokyo, Japan) by comparing published intensities in the range of 300 to 400 nm with the corresponding intensity measured in the Suntest (mean intensity = 44.1 W/m^2 measured at the start and end of the irradiation period). The calculation of the quantum yield was performed according to the method described in the OECD and ECETOC Technical Report No. 12. The quantum yield value was used for the estimation of the half-life of DCPP in an aquatic environment at different latitudes. The theoretical half-life was calculated by considering the direct phototransformation for

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 — Phototransformation in water including identity of transformation products (01)

		midday sunlight conditions, in the top millimetres of natural aquatic systems. For the calculation of the expected half-life, the computer program GCSOLAR from EPA (Version 1.20, July 1999) was used.
3.5	**Transformation products**	Six major photodegradates accounting for more than 10% of the applied radioactivity were formed during the study (M1, M4, M7, M8, M16, and M17). Besides the parent compound and the major metabolites, one fraction (M2) was detected which exceeded levels of 5% of applied. All other metabolites detected were very minor (<4.4% of applied radioactivity). Mineralization of the photodegradation products of ^{14}C-DCPP continuously increased with study progress. $^{14}CO_2$ accounted for a mean of 20.3% of the applied radioactivity on day 19. Low amounts of radioactivity were detected in the ethylene glycol traps not exceeding 2.1% of applied.
3.5.1	Method of analysis for transformation products	HPLC was used to quantify the photodegradates present in the samples (study (1)). For the identification of the major photodegradates samples were analyzed with LC/MS (study (2)).
		4 RESULTS
4.1	**Screening test**	Not performed
4.2	**Actinometer data**	See table A7_1_1_1_2-3
4.3	**Controls**	Samples of the test solution were incubated as dark control (not irradiated) to distinguish between photochemical and other reactions. No other degradation processes than photolysis could be determined.
4.4	**Photolysis data**	
4.4.1	Concentration values	The course of the concentrations of the test substance and its photodegradation products when exposed to light are summarised in table A7_1_1_1_2-4.
4.4.2	Mass balance	Total mean recoveries were 97.5 ± 3.1% of the applied radioactivity for the irradiated samples and 98.9 ± 3.0% for the dark control.
4.4.3	k^c_p	2.6074 $days^{-1}$
4.4.4	Kinetic order	Simple first order reaction kinetics were applied for the parent compound and consecutive first order reaction kinetics for the major metabolites M1, M7 and M8.
4.4.5	k^c_p / k^a_p	0.110
4.4.6	Reaction quantum yield (ϕ^c_E)	0.986 molecules degraded $photon^{-1}$
4.4.7	k_{pE}	Not reported
4.4.8	Half-life ($t_{1/2E}$)	See table A7_1_1_1_2-5
4.5	**Specification of the transformation products**	See table A7_1_1_1_2-4

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 — Phototransformation in water including identity of transformation products (01)

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test was performed according to "OECD Guideline for Testing of Chemicals, Proposal for a new Guideline, Phototransformation of Chemicals in Water – Direct and Indirect Photolysis, Draft Document August 2000".

5.2 Results and discussion

DCPP underwent very rapid photolysis with its amount decreasing from 100% of applied initially to 53.0% after just 6 hours (0.25 days) of irradiation. By day 2, it had declined to represent 1.3% of the applied radioactivity, and from day 5 onwards, it was not detectable any more. The parent compound remained stable in the dark, still representing 98.5% of the applied radioactivity at the end of the study (day 19).

Six major photodegradates accounting for more than 10% of the applied radioactivity were formed during the study (M1, M4, M7, M8, M16, and M17). M1, M7, and M8 showed a clear curve of formation and decline with maximum mean amounts of 26.3% (day 2), 19.9% (day 1) and 20.4% (day 0.25) of the applied radioactivity, respectively. At the end of the study M1 accounted to 2.3% while M7 and M8 were below detection limit. M4, M16 and M17 reached their maxima at the second last or last sampling interval, accounting for 14% (day 19), 42.9% (day 9) and 36.3% (day 19) of the applied radioactivity, respectively. Besides the parent compound and the major metabolites, one fraction (M2) was detected which exceeded levels of 5% of applied. All other metabolites detected were very minor (<4.4% of applied radioactivity).

LC/MS analysis was used for metabolite identification (study (2)). It could be shown that M1, M16 and M17 are nonhalogenated and highly polar compounds. M2 was identified as 4-chlorocatechol, M7 as monochlordihydroxy-biphenylether and M8 as a condensation product. According to these results the following degradation pathways can be assumed:

- Dechlorination most probably with following ring opening/formation of highly polar compounds
- Condensation most probably with following ring opening
- Cleavage of the ether binding and formation of chlorocatechol

Mineralization of the photodegradation products of ^{14}C-DCPP continuously increased with study progress. $^{14}CO_2$ accounted for a mean of 20.3% of the applied radioactivity on day 19. Low amounts of radioactivity were detected in the ethylene glycol traps not exceeding 2.1% of applied.

The rate of photodegradation of ^{14}C-DCPP and three of its major photodegradates, M1, M7, and M8, were described using simple first order and consecutive first order kinetics, respectively.

The results of the study demonstrate that DCPP is very rapidly photodegraded in buffered solution at pH 7 with a calculated Suntest half-life of 0.27 days. M1, M7 and M8 are further rapidly photolysed with Suntest half-lives of 1.61, 0.98, and 0.72 days, respectively.

Section A7.1.1.1.2 Annex Point IIA, VII 7.6.2.2 — Phototransformation in water including identity of transformation products (01)

5.2.1	k^c_p	2.6074 $days^{-1}$
5.2.2	K_{pE}	Not reported
5.2.3	ϕ^c_E	0.986 molecules degraded $photon^{-1}$
5.2.4	$t_{1/2E}$	See table A7_1_1_1_2-5
5.3	**Conclusion**	DCPP is very rapidly photodegraded in buffered solution at pH 7 with a calculated Suntest half-life of 0.27 days. Photodegradation involves the formation of up to 19 metabolic fractions, of which six exceeded 10% of the applied radioactivity, M1, M4, M7, M8, M16, and M17. M1, M7 and M8 are further rapidly photolysed with Suntest half-lives of 1.61, 0.98, and 0.72 days, respectively. Mineralization plays a significant role in the photolysis process of DCPP in aqueous systems. The quantum yield for the photochemical reaction was determined to be Φ = 0.986 molecules degraded $photon^{-1}$. Using the quantum yield, the half-life of ^{14}C-DCPP in aqueous systems at latitudes between 30°N and 50°N was calculated and shown to range from 0.24 to 4.86 days depending on the season. These results demonstrate that DCPP is significantly photodegraded in the aquatic environment.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Section A7.1.1.1.2 Phototransformation in water including identity of transformation products

Annex Point IIA7.6.2.2

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2010
Materials and Methods	2.2: Correction: The test solutions were not saturated with air at the beginning of the study. All glass equipment was sterilized prior to use by rinsing with an ethanol/water solution. OECD guideline 316 recommends sterilisation of glassware by autoclaving or other non-chemical methods, since chemical reagents may leave residues that could absorb in the tested nm-range and/or behave as photosensitizers. 3.4.8: Clarification: Irradiated: 0, 0.04, 0.08, 0.17, and 0.25 (study (2)) and 1, 2, 5, 9, and 19 days (study (1)) 3.4.8:Correction: For metabolite identification in study (2)^{14}C-DCPP was... 3.4.9: Addition: LC/MS analysis was used for the identification of metabolites. The limit of detection (LOD) and quantification (LOQ) values for LSC were 0.18% and 0.27%. LOD and LOQ for HPLC were 0.2% and 0.4%. 3.5.1: Addition: HPLC was used to quantify the photodegradates present in the samples (LOD, LOQ: 0.2%, 0.4%). For the identification of the major photodegradates samples were analyzed with LC/MS (LOD, LOQ: 0.18%, 0.27%).
Results and discussion	4.4.8: Substitution: 0.27 days (Suntest half-life, continuous irradiation; 1 day = 24h) The half-life of ^{14}C-DCPP in aqueous systems at latitudes between 30°N and 50°N was calculated and shown to range from 0.24 to 4.86 days depending on the season (calculated by GC SOLAR, version 1.20, U.S. EPA).. For the estimation of the environmental half lives see table A7_1_1_1_2-5.
Conclusion	5.2: Remark: The structure of the major metabolite M4 was not identified or described and is unknown. 5.3: Addition: Mineralization plays a significant role in the photolysis process of DCPP in aqueous systems ($^{14}CO_2$ accounted for a mean of 20.3% of the applied radioactivity on day 19).
Reliability	2
Acceptability	Acceptable with the amendments above
Remarks	-

Table A7_1_1_1_2-1: Description of test solution and controls

Criteria	Details
Purity of water	Bi-distilled water
Preparation of test chemical solution	The direct photolytic behavior of the test item was investigated in sterile buffer solution at pH 7. The buffer solution was prepared by diluting 296 mL 0.1 N sodium hydroxide solution with 500 mL 0.1 M monopotassium phosphate and adding bi-distilled water to reach a final volume of one liter. Its final concentration was 0.05 mol/L. The buffer solution was sterilized by autoclaving for 30 minutes at 121 °C before use. A radiolabelled application solution was prepared by dissolving an aliquot of the radiolabelled test item, supplied by the Sponsor as a solid, in 5 mL acetonitrile. After 2 minutes of ultrasonic treatment followed by vortex treatment, its radioactivity content was measured by Liquid Scintillation Counting (LSC) triplicate aliquots of 100 µL of a dilution containing 50 µL application solution in 10 mL acetonitrile. The concentration of the application solution was calculated to be 0.0959 mg DCPP/mL. First application: An aliquot of 0.7 mL of the application solution was transferred using a 1 mL Hamilton syringe to a 500 mL measuring cylinder containing 300 mL sterile buffer solution. Additional 2.8 mL acetonitrile and sterile buffer solution were then added to reach a final volume of 350 mL. Second application: A second application was performed for intervals shorter than 1 day, since the test item degraded faster than expected. An aliquot of 0.5 mL of the application solution was transferred using a 1 mL Hamilton syringe to a 250 mL measuring cylinder containing 200 mL sterile buffer solution. Additional 2.0 mL acetonitrile and sterile buffer solution were then added to reach a final volume of 250 mL.

Table A7_1_1_1_2-1: Description of test solution and controls (continued)

Test concentrations (mg a.s./L)	0.21 mg/L
Temperature (°C)	24.8 ± 0.2 °C (first application), 24.8 ± 0.1 °C (second application) and 25.1 ± 0.4 °C for the dark control
Preparation of a.s. solution	An initial stock solution of p-nitroanisole (PNA) was prepared by dissolving 15.26 mg PNA in 10 ml acetonitrile in a volumetric flask (stock solution 1 or ST1). An 1 mL aliquot thereof was transferred to a 10 mL volumetric flask and made to volume with acetonitrile. Thereafter, a 1 mL aliquot of the diluted solution was transferred in a 1 liter volumetric flask, followed by 791 mg pyridine and purified water up to the mark. The actinometer solution contained 1.51 mg PNA/L (corrected for purity of 99%, corresponding to $1 \cdot 10^{-6}$ M), 791 mg pyridine/liter (0.01 M), and 0.1% acetonitrile.
Controls	Samples of the test solution were incubated as dark control (not irradiated).
Identity and concentration of co-solvent	Not used

Table A7_1_1_1_2-2: Description of test system

Criteria	Details
Laboratory equipment	Photolysis was performed using cylindrical vessels constructed entirely of Pyrex glass and covered with quartz glass plates (to cut off radiation below 290 nm similarly to the natural sunlight cut-off by ozone), which were screwed on top of the vessel. The light was allowed to enter the solution only from the top of each vessel. For the collection of volatile compounds, the vessels were connected to an air flow-through system, with a gas flow sufficient to pass through the samples. The incoming air was passed through a water trap then through the vessels and finally through two traps to absorb any volatile compounds formed (e.g. $^{14}CO_2$). The traps were in the sequence 2 N NaOH and ethylene glycol and contained about 50 mL of each solution. All glass equipment (including the incubation vessels) was sterilized prior to use by rinsing with an ethanol/water (70:30; v/v) solution. The organic solvent was allowed to evaporate before the start of the study.
Test apparatus	The study was performed in a "Suntest CPS, Original Hanau" apparatus (Heraeus, Germany), equipped with a 1.8 kW xenon burner and an UV filter system.
Properties of artificial light source:	
Nature of light source	Xenon Burner: Max. 765 W/m^2 at max. UV filtering (lambda < 800 nm) with irradiance between 400 W/m^2 and 765 W/m^2 to a pre-set value.
Emission wavelenght spectrum	290 – 800 nm
Light intensity	The mean intensity of the artificial light penetrating the surface of the aqueous solution within the visual light spectrum 300 to 400 nm was 44.1 W/m^2.
Filters	UV filter with a 290 nm cut-off to simulate natural sunlight
Properties of natural sunlight:	Not appropriate
Latitude	Not appropriate
Hours of daylight	Not appropriate
Time of year	Not appropriate
Light intensity	Not appropriate
Solar irradiance (L_λ)	Not available

Table A7_1_1_1_2-3: Actinometer data

p-nitroanisole (PNA)	The actinometer solution contained 1.51 mg PNA/L (corrected for purity of 99%, corresponding to $1 \cdot 10^{-6}$ M), 791 mg pyridine/liter (0.01 M), and 0.1% acetonitrile.
f^{a}_{E}	0.004652 molecules degraded photon $^{-1}$
$\mathbf{k}^{a}_{p}$	23.602 days^{-1}

Table A7_1_1_1_2-4: Specification and amount of transformation products

Irradiated samples (% applied)		IRRADIATION TIME [DAYS]									
	Suntest	0	0.04	0.08	0.17	0.25	1	2	5	9	19
	Sunlight[1]	0	0.08	0.16	0.32	0.5	1.9	3.9	9.7	17.5	36.9
	Sunlight[2]	0	0.2	0.5	0.9	1.4	5.7	11.3	28.4	51.0	107.7
DCPP	A	99.0	89.0	78.6	59.3	46.5	5.5	1.4	*	*	*
	B	101.0	91.4	83.2	68.3	59.6	6.6	1.3	*	*	*
	Mean	**100.0**	**90.2**	**80.9**	**63.8**	**53.0**	**6.0**	**1.3**	*	*	*
M1 (unknown)	A	*	*	1.6	5.5	11.5	24.5	20.7	21.1	*	2.0
	B	*	*	1.2	3.1	7.3	25.6	31.9	28.1	2.7	2.5
	Mean	*	*	**1.4**	**4.3**	**9.4**	**25.0**	**26.3**	**24.6**	**1.3**	**2.3**
M2 (unknown)	A	*	2.7	5.8	7.9	7.2	7.5	2.1	*	*	*
	B	*	1.9	3.8	5.1	5.8	4.3	*	*	*	*
	Mean	*	**2.3**	**4.8**	**6.5**	**6.5**	**5.9**	**1.0**	*	*	*
M3 (unknown)	A	*	*	*	1.2	1.2	4.1	1.3	*	*	*
	B	*	*	*	1.1	0.9	4.6	2.2	*	*	*
	Mean	*	*	*	**1.2**	**1.1**	**4.4**	**1.8**	*	*	*
M4 (unknown)	A	*	*	*	*	*	4.9	6.9	15.3	16.4	20.2
	B	*	*	*	*	*	7.4	3.9	8.7	6.2	7.8
	Mean	*	*	*	*	*	**6.2**	**5.4**	**12.0**	**11.3**	**14.0**
M5 (unknown)	A	*	*	*	*	*	2.5	4.5	5.9	6.0	*
	B	*	*	*	*	*	2.9	1.6	2.5	1.7	0.9
	Mean	*	*	*	*	*	**2.7**	**3.0**	**4.2**	**3.9**	**0.5**
M6 (unknown)	A	*	*	*	*	*	2.1	7.6	3.3	4.6	*
	B	*	*	*	*	*	*	1.6	1.6	1.0	1.2
	Mean	*	*	*	*	*	**1.1**	**4.6**	**2.4**	**2.8**	**0.6**
M7 (unknown)	A	*	1.7	1.6	2.6	3.0	19.7	10.3	4.1	1.9	*
	B	*	1.3	1.4	1.9	2.8	20.1	8.1	2.0	2.4	*
	Mean	*	**1.5**	**1.5**	**2.3**	**2.9**	**19.9**	**9.2**	**3.1**	**2.1**	*
M8 (unknown)	A	*	5.6	11.1	17.5	21.9	19.1	5.4	1.6	*	*
	B	*	4.6	8.9	15.3	18.9	19.9	9.8	0.9	*	*
	Mean	*	**5.1**	**10.0**	**16.4**	**20.4**	**19.5**	**7.6**	**1.3**	*	*
M9 (unknown)	A	*	*	*	1.7	2.3	4.1	1.1	*	*	*
	B	*	*	*	1.7	2.2	2.5	1.4	*	*	*
	Mean	*	*	*	**1.7**	**2.2**	**3.3**	**1.2**	*	*	*
M11 (unknown)	A	*	*	*	3.2	4.1	*	*	*	*	*
	B	*	*	*	2.3	1.9	*	*	*	*	*
	Mean	*	*	*	**2.7**	**3.0**	*	*	*	*	*

LC/MS was used for metabolite identification. It could be shown that M1, M16 and M17 are nonhalogenated and highly polar compounds. M2 was identified as 4-chlorocatechol, M7 as monochlordihydroxybiphenylether and M8 as a condensation product.

Table A7_1_1_1_2-4: Specification and amount of transformation products (continued)

Irradiated samples (% applied)		IRRADIATION TIME [DAYS]									
	Suntest	0	0.04	0.08	0.17	0.25	1	2	5	9	19
	Sunlight[1]	0	0.08	0.16	0.32	0.5	1.9	3.9	9.7	17.5	36.9
	Sunlight[2]	0	0.2	0.5	0.9	1.4	5.7	11.3	28.4	51.0	107.7
M12 (unknown)	A	*	*	*	*	*	2.8	2.4	3.7	*	*
	B	*	*	*	*	*	1.3	*	2.3	*	*
	Mean	*	*	*	*	*	**2.0**	**1.2**	**3.0**	*	*
M13 (unknown)	A	*	*	*	*	*	*	3.4	1.9	3.1	*
	B	*	*	*	*	*	*	1.4	1.3	1.3	1.0
	Mean	*	*	*	*	*	*	**2.4**	**1.6**	**2.2**	**0.5**
M14 (unknown)	A	*	*	*	*	*	*	2.9	3.1	0.9	4.2
	B	*	*	*	*	*	*	*	2.1	1.1	2.0
	Mean	*	*	*	*	*	*	**1.5**	**2.6**	**1.0**	**3.1**
M15 (unknown)	A	*	*	*	*	*	*	5.3	3.6	6.0	2.5
	B	*	*	*	*	*	*	*	3.0	2.9	1.1
	Mean	*	*	*	*	*	*	**2.6**	**3.3**	**4.4**	**1.8**
M16 (unknown)	A	*	*	*	*	*	*	3.9	7.3	43.7	3.7
	B	*	*	*	*	*	*	6.4	11.5	42.1	12.6
	Mean	*	*	*	*	*	*	**5.1**	**9.4**	**42.9**	**8.1**
M17 (unknown)	A	*	*	*	*	*	*	11.4	16.3	2.9	38.0
	B	*	*	*	*	*	*	22.4	21.8	15.1	34.6
	Mean	*	*	*	*	*	*	**16.9**	**19.1**	**9.0**	**36.3**
M18 (unknown)	A	*	*	*	*	*	*	2.6	4.8	2.1	2.2
	B	*	*	*	*	*	*	2.7	4.4	3.3	*
	Mean	*	*	*	*	*	*	**2.7**	**4.6**	**2.7**	**1.1**
M19 (unknown)	A	*	*	*	*	*	*	2.0	*	*	*
	B	*	*	*	*	*	*	*	*	*	*
	Mean	*	*	*	*	*	*	**1.0**	*	*	*
$^{14}CO_2$	A	n.p.	<0.1	<0.1	<0.1	<0.1	0.4	1.4	4.9	8.6	16.7
	B	n.p.	<0.1	<0.1	<0.1	<0.1	0.4	1.6	6.4	13.4	23.8
Organic volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	0.3	0.5	3.7	3.8	4.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2	0.2	0.2
TOTAL	**A**	**99.0**	**99.0**	**98.7**	**98.8**	**97.7**	**97.4**	**97.2**	**100.6**	**100.0**	**93.6**
	B	**101.0**	**99.2**	**98.6**	**98.8**	**99.5**	**95.6**	**96.4**	**96.8**	**93.5**	**87.6**

n.p.: Not performed, * Not detected, A/B: Duplicate samples
[1] Summer sunlight days calculated for latitude 50°N
[2] Spring sunlight days calculated for latitudes 35°N (Tokyo)

LC/MS was used for metabolite identification. It could be shown that M1, M16 and M17 are nonhalogenated and highly polar compounds. M2 was identified as 4-chlorocatechol, M7 as monochlordihydroxybiphenylether and M8 as a condensation product.

Table A7_1_1_1_2-5: Estimated half-lives of DCPP in the environment

Theoretical lifetime (days)*	Spring	Summer	Fall	Winter
Latitude 30°N	0.296	0.239	0.451	0.762
Latitude 40°N	0.384	0.271	0.716	1.630
Latitude 50°N	0.538	0.322	1.410	4.860

* Conditions: Pure water close to the surface, longitude 10°, terrestrial type of atmosphere.

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2012): Reg.No[redacted]abel: phenole-U-C14) (Radiolabelled Diclosan) - Determination of the Ready Biodegradability in a modified CO_2-Evolution Test at aerobic conditions with radiolabelled test substance.[redacted] Report No.[redacted]5, Date: 2012-11-19	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD-Guideline No. 301 B (1992) Commission Regulation (EC) No 440/2008, C.4-C (2008)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes. Duration prolonged to 61 d (test substance and blank assays).	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled Diclosan: Reg.No[redacted](label: phenole-U-C14)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Radiolabelled test substance (label: phenole-U-C14)	
3.1.3	Purity	Radiochemical purity: 99.0 % (RHPLC) (89.8 %, determined on 27 July 2012, confirmed by radio HPLC analysis, non-GLP) Chemical purity: 92.5% (calculated)	
3.1.4	Further relevant properties	-	
3.1.5	TS inhibitory to microorganisms	No. The selected test concentration was tested in an additional inhibition control test assay with non-radiolabeled aniline. No toxic effects were observed.	
3.2	**Reference substance**	Yes, aniline (CAS No. 62-53-3) [redacted] Expiry date: 17 Aug 2012	
3.2.1	Initial concentration of reference substance	20 mg TOC/L of the reference substance	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	See Table A7_1_1_2_1-1	

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

3.3.2	Test system	The following test assays were prepared: - 2 blank control assays (BC) - 6 test substance assays (TS) - 1 inhibition control test assay (IH; run for 14 d) - 1 reference substance assay (RS; run for 14 d) The test was performed in a 1-L incubation bottles filled up to a volume of 0.5 L. A reference control with activated sludge, mineral medium and reference item; an inhibition control with activated sludge, mineral medium, reference and test item as well as a blank control containing activated sludge and mineral medium were run under the same test conditions. To account for the solvent used in the test substance assays, 2.0 mL of acetonitrile were added to the test vessels of the blank control and the reference substance assays. The solvent was removed subsequently by evaporation. The incubation bottles were connected to two serial scrubbing bottles (total volume 250 mL; 0.05 M sodium hydroxide).for the adsorption of carbon dioxide from biodegradation processes. The Total Inorganic Carbon (TIC) values or the radiolabelled carbon dioxide in the adsorption solutions of the first trap were determined and used for the calculation of the produced carbon dioxide. After each sampling the second trap was moved into first position and a new trap with fresh sodium hydroxide solution was placed into second positions. The radiolabelled carbon dioxide was determined by measurement of the radioactivity in the absorption solutions (3 aliquots from each absorption vessel). The scintillation liquid Ultima Gold TM XR from Perkin Elmer INC., was used for the measurements in liquid scintillation counters Packard – Canberra Type 2300 TR TRI CARB and Perkin Elmer 2910 TR TRI CARB.
3.3.3	Test conditions	The test conditions are described in Table A7_1_1_2_1-2 Static test at 22±2 °C, 1 L incubation bottles containing 500 mL test medium, continuously stirred, aerated with carbon-dioxide free air
3.3.4	Method of preparation of test solution	<u>Reference substance:</u> The stock solution with the reference item was prepared with deionized water. The concentration was 646.2 mg/L. Test concentrations 20 mg TOC/L of the reference item (20 mL stock solution in 500 mL test medium) <u>Test substance:</u> A stock solution with radio-labelled test substance was prepared (126.5 mg / 25 mL; = 10.38 MBq; 2 mL = 49.82E06 dpm = 49.1 µg). The

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

		solvent was acetonitrile. The test substance assays were prepared by addition of 2.0 mL of stock solution to the test vessel. The solvent was removed by evaporation. Afterwards a volume of 490 mL of mineral medium and an aliquot of 10 mL activated sludge suspension were added to obtain a concentration of approximately 95 µg/L test item.
3.3.5	Initial TS concentration	Approximately 48.6E06 dpm TAR, equivalent to approximately 95 µg/L test substance (calculated)
3.3.6	Duration of the test	61 days
3.3.7	Analytical parameter	The pH value of all incubation flasks was measured at the beginning and at the end of the test. Reference substance: carbon dioxide collected in scrubbing bottles determined as Total Inorganic Carbon via combustion/non-disperse infrared gas analysis method (Shimadzu TOC-analyser) Test item: at start of exposure: determination of total applied radioactivity (TAR) in additional test vessel; during exposure: radiolabelled carbon dioxide formed by biological processes was determined by measurement of the radioactivity in the absorption solutions (liquid scintillation counter Packard - Canberra Type 2300 TR TRI CARB and Perkin Elmer 2910 TR TRI CARB). Radioactivity in test system (CO_2, water and sludge; non-GLP)
3.3.8	Sampling	Test item: Day 0, 4, 8, 11, 14, 18, 21, 25, 34, 40, 48, 55, 61, and 62 (flask 1 and 2) Reference substance: Day 0, 4, 8, 11, 14
3.3.9	Intermediates/ degradation products	Identification of remaining DCPP or possible intermediates/degradation products via GC/MS (non-GLP) in test substance assays 1 and 2
3.3.10	Nitrate/nitrite measurement	Not applicable
3.3.11	Controls	Inhibition control, reference control
3.3.12	Statistics	Not relevant
		4 RESULTS
4.1	**Degradation of test substance**	

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

4.1.1 Graph

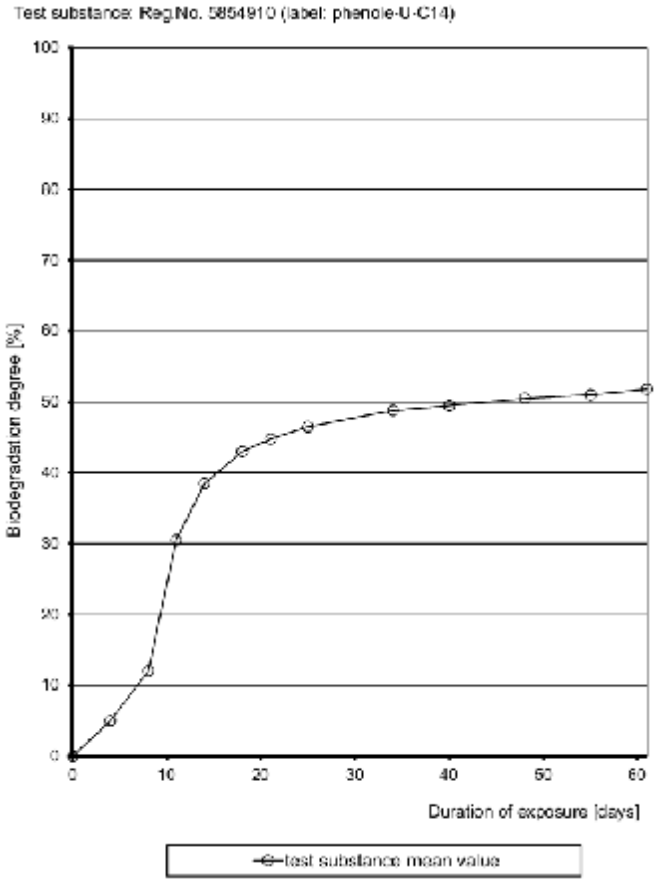


Figure 1: Biodegradation curve of test item (mean value)

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

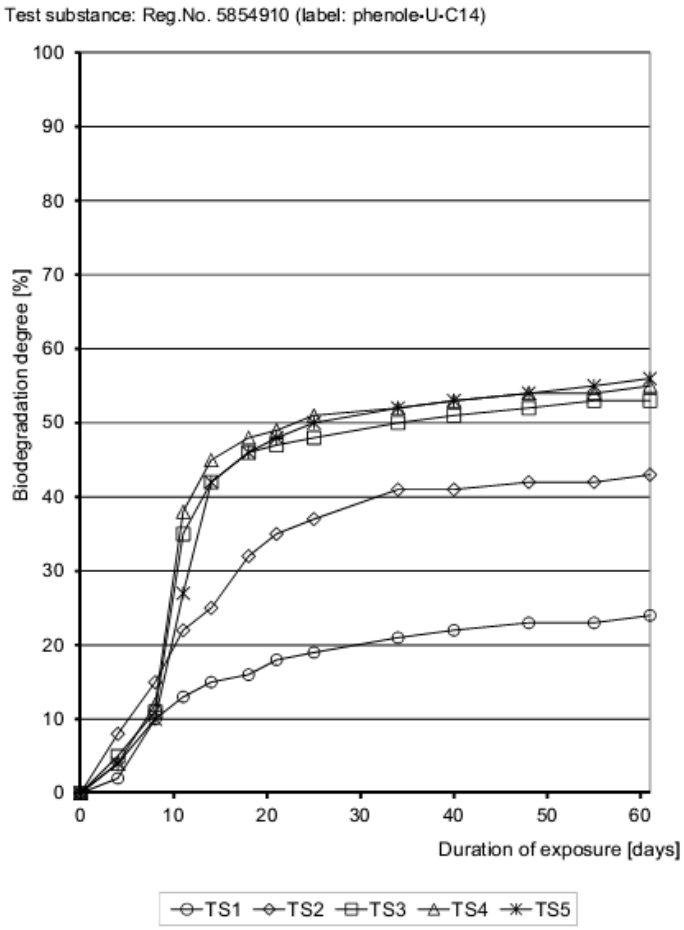


Figure 2: Biodegradation curve of all test item assays

The results are also presented in tabular form (Table A7_1_1_2_1-3).

4.1.2	Degradation	Elimination after 28 days:	40-50% (related to TAR)
		Elimination after 60 days:	52±9% (related to TAR)
		The biodegradation is shown in Table A7_1_1_2_1-3.	
4.1.3	Other observations	In the inhibition control, no inhibitory effect was observed (amount of CO_2 formed in inhibition control approximately equal to that formed in reference assay: 42.0 mg compared to 40.5 mg).	
		The biodegradation of the inhibition control is shown in Table A7_1_1_2_1-4.	
		The distribution of the radioactivity in the test system after 60 d is summarised in Table A7_1_1_2_1-5 (non-GLP).	x
4.1.4	Degradation of TS in abiotic control	Not applicable	
4.1.5	Degradation of reference substance	Biodegradation after 14 days:	> 90%
		The biodegradation of the reference substance is shown in Table	

Section A7.1.1.2.1 Ready biodegradability (01)

Annex Point IIA, VII 7.6.1.1

		A7_1_1_2_1-4.	
4.1.6	Intermediates/ degradation products	Test substance assay 1: Small proportions of DCPP were found, no metabolites were identified in a GC/MS above the background level Test substance assay 2: Neither DCPP nor any metabolites were found by GC/MS above the background level. (non-GLP results)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test on ready biodegradability of DCPP was conducted according to OECD-Guideline No. 301B (1992) and Commission Regulation (EC) No. 440/2008, C.4-C (2008). The test duration was prolonged to 61 days. The test substance was incubated with activated sludge at 22±2 °C.	
5.2	**Results and discussion**	DCPP was degraded by 40-50% within 28 days (related to TAR; *cf.* Table A7_1_1_2_1-3). After 60 days the degree of biodegradation was 52±9% (related to TAR). Based on the low degradability of test substance assay 1, this replicate is regarded as an outlier. The distribution of the radioactivity over the compartments CO_2, water and sludge of the test system revealed that water and sludge contained less than 10% TAR (Replicates 3–5). Replicate 2 had a slightly higher proportion (12%), while replicate 1 was considered to be an outlier: 30%) (Table A7_1_1_2_1-5). The sludge contained 3–5% TAR (replicates 2-5), while CO2 contained 43–55% TAR (replicate 1: 23%). The recovery of ^{14}C at test end was between 60% and 70% TAR. The low recovery at test end might be explained by small losses of $^{14}CO_2$ during the sampling processes during the exposure in combination with the low initial test concentration. The GC/MS analysis of replicates 1 and 2 showed no metabolites above background level in both replicates and only traces of DCCP in replicate 1.	
5.3	**Conclusion**	Overall, it can be concluded that DCPP is biodegradable under aerobic conditions.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No.	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	November 2012
Materials and Methods	Adopt applicant's version.
Results and discussion	Other observations As the exposure period is given with 61 days, the distribution of the radioactivity in the test system occurred after 61 days.
Conclusion	Defencencies: *Add*: Total ^{14}C found were only between 60 to 70% for the test substance assays after 61 days, indicating loss of substance.
Reliability	1
Acceptability	Acceptable for environmental risk assessment
Remarks	Direct comparison of the test substance assays to inhibition control test assay and reference substance control are not possible, as only for test substance assays radioactivity was determined.

Table A7_1_1_2_1-1: Inoculum

Criteria	Details
Nature	Activated sludge
Species	No data
Source/Sampling site	Collected from a municipal wastewater treatment plant (Mannheim, Germany).
Sampling date	2012-04-10
Pretreatment	Activated sludge was sieved by a finely woven mesh (1 mm) and pre-aerated for about 48 h at 22±2 °C. At the day of exposure the sludge was washed one time with tap water. The suspension was allowed to settle. The supernatant was discarded and the remaining sludge was filled up with tap water to 1.5 g/L dry weight. The pH value of the activated sludge was 7.3.
Initial cell concentration	Not reported

Table A7_1_1_2_1-2: Test conditions

Criteria	Details
Composition of medium	The used mineral medium complies with the test guideline OECD 301B. It was prepared from the following solutions. The mineral salts for each of the following stock solutions were dissolved in 1 L of distilled water. Stock solution A: 8.5 g of potassium dihydrogen phosphate, 21.75 g of di-potassium hydrogen phosphate, 33.4 g of di-sodium hydrogenphosphate dihydrate and 0.5 g of ammonium chloride. The pH value of this solution was adjusted to pH 7.4. Stock solution B 36.4 g of calcium chloride dehydrate Stock solution C 22.5 g of magnesium sulphate heptahydrate Stock solution D 0.25 g of ferric chloride hexahydrate 10 mL of solution (A) and 1 mL of solutions (B), (C) and (D) were added per 1 L distilled water to form the test medium. The pH was adjusted to 7.4±0.2.
Additional substrate	No
Test temperature	22±2 °C
pH	Start of exposure: ; 7.3 end of exposure: 7.1
Aeration of dilution water	See above
Suspended solids concentration	Activated sludge concentration: 30 mg/L suspended solids dry weight The concentration was obtained by adding 10 mL of the pre-treated sludge (1.5 g/L SS) per 500 mL test medium.
Concentration of inoculum	See above
Other relevant criteria	None

Table A7_1_1_2_1-3: Biodegradability of test item (based on total applied radioactivity)

Duration of exposure [d]	Degree of biodegradation [%]					
	TS1*	TS2	TS3	TS4	TS5	TS mean value
0	0	0	0	0	0	0
4	2	8	5	4	4	5
8	10	15	11	12	10	12
11	13	22	35	38	27	31
14	15	25	42	45	42	39
18	16	32	46	48	46	43
21	18	35	47	49	48	45
25	19	37	48	51	50	47
34	21	41	50	52	52	49
40	22	41	51	53	53	50
48	23	42	52	54	54	51
55	23	42	53	54	55	51
61	24	43	53	55	56	52

* Remark: The result of test vessel TS1 as considered as an outlier and therefore not included in the evaluation.

Table A7_1_1_2_1-4: Carbon dioxide formed and biodegradation (blank control, reference item and inhibition control)

Duration of exposure [d]	Formed carbon dioxide amount in the test vessels					Degree of biodegradation	
	[mg CO_2/test vessel]			[mg CO_2 summarised]		[% CO_2/ThCO_2]	
	BC mv	RS	IH	RS	IH	RS	IH
0						0	0
4	16.1	35.6	31.7	19.5	15.6	53	43
8	11.3	26.9	25.3	35.1	29.6	96	81
11	12.8	18.2	22.1	40.5	38.9	110	106
14	12.1	12.1	15.2	40.5	42.0	110	114

Table A7_1_1_2_1-5: Distribution of radioactivity in test system after 60 d (in % of total applied radioactivity; non-GLP)

Compartment	CO_2	Water	Sludge (measured)	Total ^{14}C found
Replicate 1	23 %	30 %	17 %	70 %
Replicate 2	43 %	12 %	5 %	60 %
Replicate 3	52 %	7 %	4 %	61 %
Replicate 4	54 %	8 %	5 %	67 %
Replicate 5	55 %	8 %	3 %	66 %

Table A7_1_1_2_1-6: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or ThCO_2	-	**X**
Pass values reached within 10-d window (within 28-d test period)	-	**X**
Criteria for validity		
Percentage of removal of reference substance reaches pass level by day 14	**X**	-
The formed amount of carbon dioxide in the inhibition control should be approximately equal with the amount of the formed carbon dioxide in the reference substance assay after 14 days:	**X**	-

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2002). Ready biodegradability of [redacted] (Manometric respirometry test), [redacted] report number: [redacted] report date: 04 Nov 2002 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes - OECD Guideline 301 F - EU directive 92/69/EEC, C.4-D	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] Me-O-DCS, methoxydiclosan	
3.1.1	Lot/Batch number	[redacted] (expiration date: 30 Sep 2010)	
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.3	Purity	>99%	
3.1.4	Further relevant properties	- Solubility: 1-10 mg/L - White powder - ThOD: 0.157 mg/L (without nitrification)	
3.1.5	Composition of Product	Not applicable	
3.1.6	TS inhibitory to microorganisms	No	
3.1.7	Specific chemical analysis	Gas Chromatography - GC/MSD (HP5890/HP5971) in SIM mode - Split-less mode at 250 °C injector temperature - Column: Optima delta 6 (30 m, 0.25 mm ID; 0.25 µm film); temperature: 80 °C for 1 min, increased to 140 °C (5 °C/min), increased to 230 °C (15 °C/min), increased to 280 °C (6 °C/min); hold time: 1.67 min; run time: 29 min - GC/MSD transfer line: 260 °C, constant - Quantification: internal standard multi-point calibration curve	

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

		- Detection limit for all reference compounds: ≤ 2.5 µg/L	
		Sample preparation (extraction): SPE (SPE cartridge: Chromabond EASY 6 mL/500 mg)	
3.2	**Reference substance**	Yes (sodium benzoate)	
3.2.1	Initial concentration of reference substance	100 mg/L according to guideline	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	Mixture of polyvalent bacteria (activated sludge) collected on 27 May 2002 from the aeration tank of a domestic sewage treatment plant (sampling site: ARA Pro Rheno Basel, Switzerland)	
		Bacteria concentration in test medium: 26 mg/L suspended solids	
3.3.2	Test system	See table A7_1_1_2-3	
3.3.3	Test conditions	See table A7_1_1_2-4	x
3.3.4	Method of preparation of test solution	A stock solution (2 mg/L) was prepared by dissolving 20.1 mg test item in 10 L of distilled water with addition of 5 mL NaOH (5N) one day before test start. The stock solution was continuously stirred until test start.	
		To obtain the test solution (100 µg/L), 100 mL of the stock solution were further diluted by mixing with distilled water (2 L total volume). 250 mL of this solution were given to the test vessels. Afterwards the stock solutions of the test medium (2.5 mL A, 0.25 mL B, C, and D) and 2.5 mL of activated sludge were added directly to the test vessels.	
3.3.5	Initial TS concentration	100 µg/L	
3.3.6	Duration of test	28 d	
3.3.7	Analytical parameter	Test item: Gas chromatography (elimination; different method due to low test concentration)	
		Reference item: Oxygen consumption (BOD)	
3.3.8	Sampling	At test start; after 14, 26 and 28 d	
3.3.9	Intermediates/ degradation products	Identified	
		GC/MSD (4-chlorocatechol; 4-chloro-2-methoxy-1-phenol; 2-, 3-, and 4-chloroanisole; 2-, 3-, and 4-chlorophenol; see 3.1.7)	x
3.3.10	Nitrate/nitrite measurement	No	
3.3.11	Controls	- Inoculum control (inoculum + test medium)	
		- Procedure control (inoculum + test medium + reference item)	
		- Abiotic sterile control (test medium + test item + sterilising agent (1 mL 1% $HgCl_2$) without inoculum)	
		- Toxicity control (inoculum + test medium + test item + reference item)	
3.3.12	Statistics	Elimination of methoxydiclosan	

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

$$E_t\,(\%) = 1 - \left[\frac{DCS_t - DCS_{bl\_t}}{DCS_{AW}}\right] * 100$$

E_t = elimination (%) at time t

DCS_t = value of the test item at time t (µg/L)

DCS_{bl_t} = value of the blank control at time t (µg/L)

DCS_{AW} = value of the test item at test start (µg/L)

Similar formulas may be used for metabolites.

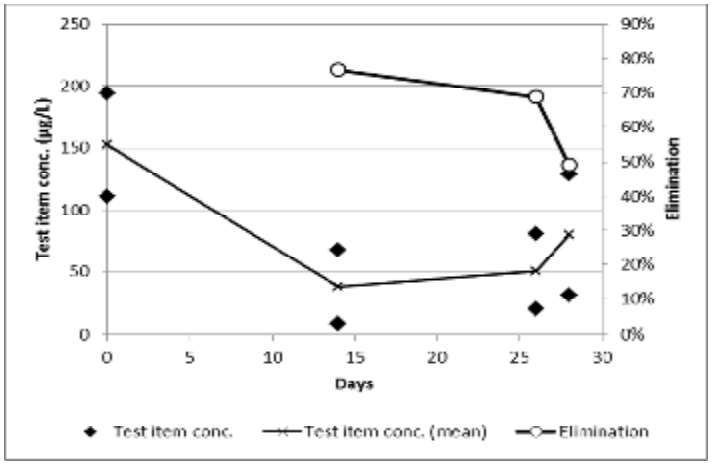
Elimination was recalculated from the data given in the report. In contrast to the study report, where the two sampled vessels per sampling time were arranged as two replicates, a mean value was formed from both measurements of the same day. In a second step the elimination was calculated.

The degradation of the reference item as well as the degradation in the controls was based on BOD and ThOD according to the guideline.

4 RESULTS

4.1 Degradation of test substance

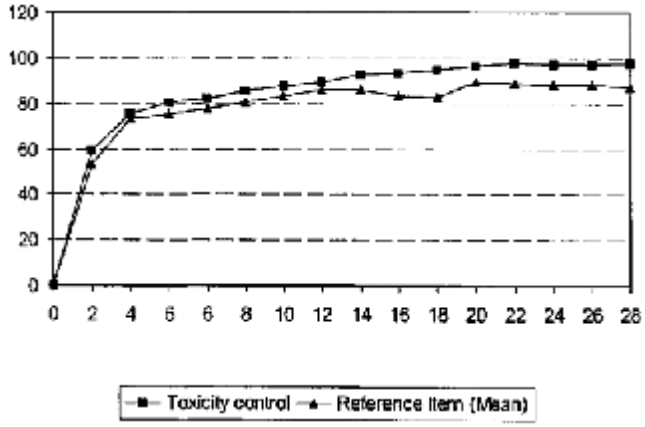
4.1.1 Graph



4.1.2	Degradation	Elimination: 49% after 28 d	x
		See table A7_1_1_2-5.	x
4.1.3	Other observations	Biodegradation in toxicity control: 93% after 14 d	
		See table A7_1_1_2-6.	
4.1.4	Degradation of TS in abiotic control	1% after 28 d	

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

4.1.5	Degradation of reference substance	 x-axis = incubation period in days; y-axis = degradation (%) 86% after 14 d 87% after 28 d	
4.1.6	Intermediates/ degradation products	None of the possible degradation products was detected (≤ 2.5 µg/L, LOD). Diclosan was above the concentration limit in two samples (day 14 – vessel 23: 4.3 µg/L; day 26 – vessel 21: 5.0 µg/L).	x

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The ready biodegradability of M-DCPP was studied in a manometric respirometry test according to OECD 301 F. Activated sludge from a sewage treatment plant receiving predominantly domestic sewage was used as inoculum. The test assays (test volume = 250 mL) were incubated at 22.0 °C in the dark for a maximum duration of 28 days. The test item concentration was aimed at 100 µg/L. Therefore, the degradation/elimination of the test item was monitored via GC/MSD. The test concentration of the reference item was 100 mg/L. The biodegradation of the reference item as well as the controls were monitored via respirometry.	
5.2	**Results and discussion**	The substance was eliminated to 49% after 28 days of incubation. The results of the chemical analysis showed varying concentrations with an irregular trend of elimination. Over the course of the incubation period, the test item concentrations decreased, but the variation between the individual measurements was high. On the last sampling time (28 d), one measurement (129 µg/L) was clearly above the initial test concentration of 100 µg/L. The results have been verified by re-analysis of the samples. The variability and irregularity was explained in the study report by the poor water solubility of the test item. The test item concentration was aimed to be 100 µg/L. Regarding the analytical determinations at test start, this concentration was only achieved in one vessel, while the other was twice as high (194 µg/L).	x
5.3	**Conclusion**	The test was considered valid because: - The degradation of the reference item at day 14 reached the pass level of 60%. - The degradation of the toxicity control at day 14 was > 25%.	

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

- The abiotic degradation of the sterile control at day 28 was not >10%.
- The oxygen consumption of the inoculum control was not greater than 60 mg/L (the value of the oxygen consumption i s normally 20-30 mg/L).
- The pH value at the end of the test was inside the range of 6.0-8.5.
- Regarding the difference of extremes at the plateau phase of the test item degradation curve, this criterion cannot be judged since this phase was not achieved by the test item.

Based on the calculated elimination of 49%, the test item is not readily biodegradable, but should be assessed as moderately degradable

The lowest test item concentration was 8.5 µg/L (day 14) and at test end 31 µg/L. Therefore depending on the initial concentration ready biodegradability could have been achieved. The preparation of the stock solution was probably not appropriate to achieve a homogenous test solution.

5.3.1	Reliability	2
5.3.2	Deficiencies	Yes

- Test item concentrations at test start with high variability between test vessels. Only one vessel was close to the aimed test concentration of 100 µg/L. The stock solution probably should have been filtered prior to addition to test medium in order to achieve a homogeneous solution and to remove any undissolved particles. This could have reduced the variability between the test vessels at test start.
- The irregular elimination trend of the test item could also be a result of the poor water solubility and the high variability of the test item concentration at test start. The calculated elimination should therefore be used with caution.

Section A7.1.1.2.1 Ready Biodegradability

Annex Point IIA7.6.1.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	2.3: Neither CO_2 nor O_2-development measured regarding test substance.
Materials and Methods	3.3.3 Table A7_1_1_2-4: Inclusion: "The pH value at the end of the test was not measured for the vessels containing the test item as these test vessels were immediately sent to the analytical laboratories without any treatment." 3.3.9: Inclusion of "Diclosan".
Results and discussion	4.1.2. + Table A7_1-1-2-5: The recalculation of the % elimination could not be followed: Substitute the values gained with those obtained in the original study: % Elimination after 14 d: 75% % Elimination after 26 days: 67% % Elimination after 28 days 48% 4.1.6: Substitute concentration with detection limit.
Conclusion	5.2.: Substitute 49% with 48%.
Reliability	2
Acceptability	Acceptable for environmental risk assessment with stated limitations
Remarks	The study design was not appropriate to prove mineralisation of test substance, as neither CO_2 or O_2 are measured in the setup for the test substance. Just elimination is measured by a substance specific method, not including radio-labelling. Hence, no data on mineralization and ultimate degradation can be obtained from this test. Throughout the test, the elimination of Methyl-DCPP was very irregular and varied in the range from 94.4% to 15.4%. No clear elimination rate could be determined – possibly due to bad water solubility of the test item.

Table A7_1_1_2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
DOC Die-Away-Test	C.4-A	301A	ready
CO_2 Evolution-Test (Modified Sturm Test)	C.4-C	301B	ready
Modified OECD-Screening-Test	C.4-B	301E	ready
Manometric Respirometry	C.4-D	301F	ready
MITI-I-Test	C.4-F	301C	ready
Closed-Bottle-Test	C.4-E	301D	ready
Zahn-Wellens-test	C.9	302B	Inherent
Modified MITI-Test (II)	-	302C	Inherent
Modified SCAS-Test	C.12	302A	Inherent
Simulation Test with activated Sewage (Coupled Units-Test)	C.10	302A	Simulation Test[1)]

[1)] Test for the determination of the ultimate degradation of test material under conditions which simulate the treatment in an activated sludge plant

Table A7_1_1_2-2: Inoculum / Test organism

Criteria	Details
Nature	Activated sludge
Species	Mixture of polyvalent bacteria
Strain	Not applicable
Source	Sewage treatment plant treating predominantly domestic sewage
Sampling site	ARA Pro Rheno Basel, Switzerland
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	Activated sludge washed 4 times with tap water and the last time with test medium
Pretreatment	Determination of dry weight of suspended solids. 2-3 g/L suspended solids mixed with test medium. Sludge aerated until use. pH = 7.4
Initial cell concentration	Pre-treated sludge: 2.6 g/L suspended solids

Table A7_1_1_2-3: Test system

Criteria	Details
Culturing apparatus	500 mL conical vessels containing 250 mL test medium, continuously stirred
Number of culture flasks/concentration	Test item:8 Inoculum control: 4 Procedure control (reference item): 1 Abiotic sterile control (without inoculum, with sterilising agent, $HgCl_2$): 1 Toxicity control (test and reference item): 1
Aeration device	Not applicable
Measuring equipment	Test item: Gas chromatography Reference item: Oxygen consumption (Voith Sapromat apparatus)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_1_1_2-4: Test conditions

Criteria	Details
Composition of medium	Mineral medium according to OECD TG 301
Additional substrate	No
Test temperature	22.0 °C
pH	Start of test: - Initial: 7.5 to 8.6 - Corrected: 7.3 to 7.6 End of test: 7.6 to 8.0
Aeration of dilution water	No
Suspended solids concentration	26 mg/L suspended solids
Other relevant citeria	Continuously stirred

Table A7_1_1_2-5: Analytical determinations and elimination of test item (results from re-analysis of samples in brackets)

Time (d)	Test item (µg/L)				Elimination (%)
	Blank	Measurement 1	Measurement 2	Mean	
0	≤2.5	194 (205)	111 (119)	153	-
14	≤2.5	67.7 (71.5)	8.5 (8.3)	38	77%
26	≤2.5	19.7 (22.0)	80.6 (85.7)	50	69%
28	≤2.5	31.0 (38.0)	129 (131)	80	49%

Table A7_1_1_2-6: Biodegradation of controls

Incubation	Oxygen consumption (mg/L)				Biodegradation (%)	
Days	Blank	Procedure control	Abiotic control	Toxicity control	Procedure control	Toxicity control
2	5	94	1	104	53	59
4	8	130	1	134	73	75
5	9	135	1	143	75	80
6	9	139	1	146	78	82
8	9	143	1	152	80	86
10	9	148	1	155	83	87
12	9	153	1	158	86	89
14	9	153	1	164	86	93
16	14	153	1	170	83	93
18	15	153	1	173	83	95
20	15	164	1	176	89	96
22	16	164	1	179	89	98
24	17	164	1	179	88	97
26	17	164	1	179	88	97
28	19	164	1	182	87	98

Table A7_1_1_2-7: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		X (49%)
Pass values reached within 10-d window (within 28-d test period)		X
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%		(X)
Percentage of removal of reference substance reaches pass level by day 14	X	

5.3.2.1 Criteria for poorly soluble test substances	5.3.2.2	5.3.2.3
5.3.2.4	**5.3.2.5**	**5.3.2.6**
5.3.2.7	**5.3.2.8**	**5.3.2.9**

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]: Inherent biodegradability of [redacted] (Zahn-Wellens/EMPA-test). [redacted] Test No. [redacted] Date: 2001-02-02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline 302 B (1993), Zahn-Wellens test and 87/302/EEC, Part C (1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Test substance concentration 100 µg/L (due to antimicrobial properties) Analysis of test substance: GC/MSD (concentration too small for DOC analysis)	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance DCPP [redacted]	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	Not given	x
3.1.4	Further relevant properties	-	x
3.2	**Reference substance**	Yes; Diethylene glycol (Fluka no. 32160) Analytical report: -[redacted] - 2-Chlorophenol: Supelco JNC Lot. LA 16486 - 3-Chlorophenol: Fluka 25840 95% - 4-Chlorophenol: Fluka 25850 99% - Methoxy-benzene (Anisole): Aldrich 4-Chloranisol 99% - Methoxy-DCPP, synthesised from [redacted]	
3.2.1	Initial concentration of reference substance	153.6 and 153.9 mg/L DOC of the reference substance	

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_1_2_2-1.	
3.3.2	Test system	A mixture of a total test volume of 2 L each containing test substance, mineral medium and activated sludge was agitated and aerated at 20-25 °C in two glass vessels (brown glass) in the dark for 28 days.	x
		A reference control running (two glass vessels) containing activated sludge, mineral medium and the readily biodegradable reference substance and an inoculum control (one glass vessel) containing activated sludge and test substance were run under the same conditions.	
		The biodegradation process of the test substance was monitored by analytical determination of the test substance concentrations. The degradation of the reference substance was followed by determination of the dissolved organic carbon (DOC) concentration.	
		The concentration of dissolved oxygen was checked at regular intervals to ensure that the oxygen did not fall below 2 mg/L.	
		The pH was checked and adjusted to pH 7.0-8.0 with NaOH if necessary.	
		The test system is described in Table A7_1_1_2_2-2.	
		Analytical report: a) Water sample preparation	
		Prior to analysis, the samples were removed from the refrigerator and 11 drops of conc. phosphoric acid were added to reach a pH value of < 2. After the samples are thawed, 555 ng of $^{13}C_6$-Triclosan was spiked to the samples as internal standard.	
		b) SFE thimble preparation and blank control of SFE thimbles:	
		SFE thimbles (7 mL volume) were packed with glass wool and Tenax as adsorbent . The thimbles were spiked with 55 ng $^{13}C_6$-TCS (internal standard) and extracted with SFE. The SFE extracts of the thimbles were derivatised with N,N-diethyl-trimethylsilylamine and analysed with GC/MSD. After control analysis the inlet side of all thimbles were marked (flow direction) and the thimbles were stored, wrapped in aluminum foil.	
		c) DCPP sampling (water samples)	
		Prior to sampling the thimbles were unpacked, the analytical thimble caps were removed and replaced by the “sampling caps“. The thimbles were connected with a restrictor valve and the “vacuum system“ with some tubes and pipes. The “vacuum system“ consists of an exsiccator, which is evacuated during the whole sampling procedure with an appropriate vacuum pump unit and a vacuum gauge to maintain the pressure.	
		At the inlet side of the thimble a glass funnel was mounted and the sample was transferred into the funnel. A flow of some mL/min was	

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

adjusted at the restrictor valve to suck the sample into the thimble packing. After the sample 3 x 20 ml water were pipetted into the funnel and sucked through the thimble packing.

At the end of sampling some air was sucked trough the thimble packing. The thimbles were removed from the sampling device and the sampling caps were exchanged for the analytical thimble caps (the same as used in blank control analysis). The thimbles were wrapped in aluminium foil and stored in a refrigerator at approx. -20 °C. The whole thimbles were freeze dried, in a lyophilisator unit for approx. 18 h. Thereafter the thimbles were extracted with SFE.

d) DCPP sampling (sludge samples)

The sludge samples were extracted and worked up analogous to the water sample, with the exception, that the sludge particles were filtered during the transfer of the sample to the thimble. Therefore a filter device was connected with the thimble between the funnel and the inlet side of the thimble. The sludge suspension was pipetted into the funnel and sucked through the filter and the thimble packing. After the sampling the filter was removed from the filter device and inserted into the thimble (at the inlet side). Thereafter the thimble was lyophilised, SFE-extracted and the extracts were analysed identically as described for the water samples.

e) SFE-apparatus and extraction conditions

The extraction of the thimbles was carried out with a SFE apparatus under the following operating conditions:

HP 7680T SEE module, controlled by the SFE ChemStation software version B.01.02

- extraction medium: pure SFE grade CO_2
- extraction chamber: temperature 80 °C
- static modifier: 60 µL formic acid
- extraction steps: 379 bar, 15 min static followed by 25 min dynamic CO_2 flow rate (dynamic step) 1 mL/min
- nozzle temperature 45 °C
- trap (material) octadecyl-silica (ODS trap purchased by Hewlett-Packard)
- trap temperature (sorption step): 10 °C
- trap rinsing solvent: 1.5 mL hexane (p.A)
- trap volume compensation: 1.0 mL ± 0.1 mL
- trap rinsing flow: 1 mL/min
- trap temperature (rinse step): 20 °C

The final hexane extracts were evaporated under N_2 to a volume of about 0.5 ml and approx. 0.5 mL derivatising reagent N,N-diethyl-trimethylsilylamine were added.

In addition to the “Study Specific Operating Procedure“ a “reagent blank“ was measured and the content of the investigated compounds were determined. Therefore, Toluene and the derivatising reagent N,N-diethyl-trimethylsilylamine were mixed (approx. 1/1) and PCB 52 as injection standard was added. This extract was measured and quantified identically like the samples. Two results of this “measurement blank“

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

		samples were used in the discussion of the detection limit and the significance level of the data. f) GC/MSD parameters: The analysis of the derivatised extracts was carried out with a GC/MSD (HP 5890 GC coupled with a HP 5970 mass selective detector) without further clean up. 1 µL of the extracts were injected via autosampler in a split/splitless injector at 250 °C in the splitless mode (1 min). A DB5-type column (30 m, 0.3 mm i.d., 0.25 µm film) and helium as carrier gas was used. The column temperature was held constant at 60 °C for 1 min after injection, then increased to 180 °C at a rate of 5 °C/min, followed by an temperature increase of 15 °C/min to 280 °C (held at 280 °C for at least 10 min). The transfer line temperature to MSD was const. 260 °C. Data acquisition was done in SIM mode.
3.3.3	Test conditions	The test conditions are described in Table A7_1_1_2_2-3.
3.3.4	Method of preparation of test solution	Test substance: Immediately before the start of the test, a stock solution of 2 mg test substance in 1 L distilled water was prepared. To obtain the concentration of 100 µg/L test substance, 100 mL of the stock solution were added to the test vessel and filled up to 2 L with test medium including activated sludge. Reference substance: The day prior to the start of the test, the stock solutions were prepared. 660.0 mg and 659.2 mg reference item in 1 L distilled water. The stock solution was diluted 1:1 with test medium including activated sludge to get the final test concentration of 153.6 and 153.9 mg/L DOC for the reference substance. The stock solution was prepared as dose as practically possible to the start of the test.
3.3.5	Initial TS concentration	100 µg/L of the test substance
3.3.6	Duration of the test	28 days
3.3.7	Analytical parameter	Reference substance: DOC determination Test item + metabolites: GC/MSD-method
3.3.8	Sampling	0, 3 hours, 1 day, 5 days, 7 days, 14 days, 21 days, 27 days and 28 days
3.3.9	Analytical methods	During the test, samples of each test flask were taken at certain time intervals. The samples were centrifuged, the supernatant and the solid phase were kept in separate vessels at -18 °C to -25 °C until analysis. The sample (wet) weight of water and sludge samples was determined. The concentrations of the test item were analytically quantified in the analytic report (GC/MSD: HP 5890 GC coupled with HP 5970 mass selective detector). For details on the GC/MSD see Table A7_1_1_2_2-4. Quantification was performed according to the isotope dilution method with individual response factors for each compound.

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

		During the test the DOC concentration in each reference vessel was determined at certain time intervals. Prior to the DOC-determinations the particles of the activated sludge were separated from the samples by centrifugation (4000 rpm for 20 minutes). DOC determination of reference substance: TOC/DOC Analyser SHIMADZU TOC 500 The analytical method and the measuring equipment are summarized in Table 7_1_1_2_2-2. The concentration of DCPP and possible degradation products in the water phase and in the solid phase (sludge) was calculated based on the absolute content and the sample weight as specified in Table 7_1_1_2_2-6. The following formulas were used for the calculation of the concentrations of Diclosan and degradation products. Since no data were available regarding the dry matter content of the sludge samples, the analysed absolute contents were related to the weight of the complete sample (water + sludge) and the initial concentration of the sludge which was given as dry weight. $\text{Conc.}_{\text{water}}\,[\mu g/L] = \frac{\text{absolute content [ng]}}{(\text{sample weight water})[g]}$ $\text{Conc.}_{\text{sludge}}[\text{mg/kg dw}] = \frac{\text{absolute content [ng]}}{(\text{sample weight water + sludge})[g] \cdot \text{sludge conc.}\,[\frac{g}{L}]}$ $\text{sludge conc. (initial)} = 0.49\ \text{g dry weight/L}$	
3.3.10	Intermediates/ degradation products	- 2-Chlorophenol - 3-Chlorophenol - 4-Chlorophenol - Methoxy-benzene (Anisole) - Methoxy-DCPP	x x
3.3.11	Nitrate/nitrite measurement	Not applicable	
3.3.12	Controls	Inoculum control, Reference control	
3.3.13	Statistics	Not relevant	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	The results are presented in tabular form (Table A7_1_1_2_2-7)	x
4.1.2	Degradation	Elimination after 3 hours: 39.5%* Elimination after 24 hours: 71.3%* Total elimination after 28 days: 100%* *average of two tests running in duplicate (GC-MSD), results taken from the analytic report. The biodegradation of DCPP is shown in Table A7_1_1_2_2-5.	x, x, x

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

4.1.3	Other observations	-	
4.1.4	Degradation of TS in abiotic control	Not reported	
4.1.5	Degradation of reference substance	Biodegradation after 28 days: 100%* *average of two tests running in duplicate The biodegradation of the reference substance is shown in Table A7_1_1_2_2-8.	x
4.1.6	Intermediates/ degradation products	2-Chlorophenol, 3-Chlorophenol, 4-Chlorophenol and Methoxy-benzene (Anisole) could not definitely be quantified or data were considered as unreliable. Methyl-DCPP was quantified in the sample extracts (sludge) in low amounts and is considered to be slowly but completely biodegradable. The absolute contents of the compounds are summarised in Table A7_1_1_2_2-6. The concentrations of the compounds are summarised in Table A7_1_1_2_2-7.	x x
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The inherent biodegradability of DCPP was determined according to OECD guideline 302 B. A Zahn-Wellens/EMPA test with the test substance at a concentration of 0.1 mg/L was conducted for a test period of 28 days. The biodegradation process of the test substance was monitored by analytical determination of the compound’s concentration.	x x

Section A7.1.1.2.2 Inherent biodegradability

Annex Point IIA, VII 7.6.1.2

5.2	**Results and discussion**	Elimination of DCPP was > 99% after 28 days. The reference substance revealed a degradation of 100% after 28 days. Regarding possible degradation products, it should be noted that 2- and 3-chlorophenol were below the detection limit in water and sludge sample throughout the test. In case of 4-chlorophenol, the concentrations were either below the detection limit or were not significant in the water phase. The concentration in the sludge samples was below the detection limit or not significant most of the time. In only a few cases (on day 5 to 14) significant amounts of 4-chlorophenol could be detected, but cannot be considered relevant as they were below the 5% level. In case of 4-chloroanisole most samples showed concentrations which were below the detection limit or not significant in water and sludge samples. In case of significant values, these were still low and just slightly above the critical level and below the 5% level marking relevant metabolites. In the water phase, methyl-DCPP showed a maximum concentration of 0.85 µg/ and 1.21 µg/L on day 7 in vessels 4 and 5. This corresponds to the maximum concentrations in the sludge samples where the maximum concentrations were detected on day 7 (vessel 4) and day 14 (vessel 5). After that a steady decline can be noted. Starting on day 14 (vessel 4) and day 21 (vessel 5), the concentrations were not significant any more. Methyl-DCPP is not a relevant degradation product as it stayed below the 5% level throughout the test in water and sludge samples. In sludge samples the maximum was 4% based on the measured DCPP concentration in the initial sample. In case of the water samples, this fraction was even lower (max. 1%).
5.3	**Conclusion**	It can be concluded that DCPP is inherently biodegradable.
5.3.1	Reliability	1
5.3.2	Deficiencies	No abiotic control; test parameter was the dissipation of DCPP (primary degradation) and not the formation of CO_2 (ultimate degradation).

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2011 + August 2013
Materials and Methods	3.1.3: Addition: >99%
	3.1.4: Addition: Solubility in water: 19.5 mg/L (determined in RCC Project 712012)
	Vapour pressure: 1.2*10-06 Pa at 25 °C
	3.3.2: Exchange “in the dark” by “in diffuse daylight”.
	3.3.10: Addition: For the following anticipated metabolites water and sludge samples were analysed:
	3.3.10: Exchange “Methoxy” by “Methyl”.
Results and discussion	*4.1.1:* Exchange the title of table A7_1_1_2_2-7“Degradation of DCPP and the reference substance” by “Elimination of DCPP and degradation the reference substance”.
	4.1.2: Exchange the term “biodegradation” by the term “elimination”.
	4.1.2: Addition: Additionally, the elimination of DCPP within 28 days was observed in the sludge samples, although slower than in the water samples.
	4.1.2: Exchange “Table A7_1_1_2_2-5” by “Table A7_1_1_2_2-7”.
	4.1.5: Exchange the term “biodegradation” by the term “elimination”.
	4.1.6: Exchange “2-Chlorophenol, 3-Chlorophenol, 4-Chlorophenol and Methoxy-benzene (Anisole) could not definitely quantified or data was considered as unreliable.” by
	“2-Chlorophenol and 3-Chlorophenol could not be detected in the samples. 4-Chlorophenol and Methoxy-benzene (Anisole) could be quantified in a low amounts in some samples. Nevertheless, these values are considered to be of very limited informative value”
	4.1.6: Exchange “Methyl-DCPP was quantified in the sample extracts (sludge) in low amounts and is considered to be slowly but completely biodegradable.” by
	“Methyl-DCPP could be quantified in the water samples with a maximum on day 7. In the two sludge samples Methyl-DCPP could be quantified with a maximum on day 7 and 14, respectively.”

Conclusion	5.1: Insert term “primary” after term “inherent”. 5.1: Exchange the term “biodegradation” by the term “elimination”. 5.1: ad Table A7_1_1_2_2-5: Change “Yes (for the removal of DCPP determined by GC/MSD)1“ in the box for“20% removal (DOC or COD)” to “No”. Change “Yes (for the removal of DCPP determined by GC/MSD)1)*“ in the box for” Pass values reached within 10-d window (within 28-d test period)”. Change “Yes (for the removal of DCPP determined by GC/MSD)1) in the box for “Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)” to “Removal of DCPP after 3 hours already 39.5%, Elimination after 24 hours 71.3%: These data cannot exclude adsorption processes”. Deletion of the boxes “Percentage of DOC-removal of reference compound ≥ 40% within 7 days and ≥ 65% within 14 days, Average residual amount of test compound in blank tests ≥ 40% (OECD 302 C)” and the corresponding box with “Yes”. Deletion of the footnotes: “1) Test parameter was the dissipation of DCPP (primary degradation) and not the formation of CO2 (ultimate degradation), *)10-day value not available; however the pass level was reached before 10 days of incubation”. 5.3.2: Addition: Adsorption processes cannot be excluded. No biodegradation curve could be presented in the test report as no DOC or COD was measured for the test conducted with the test item the test is not able to show ultimate biodegradation of DCPP .
Reliability	2
Acceptability	Acceptable for environmental risk assessment with amendments given above. As no DOC or COD was measured for the test conducted with the test item the test is not able to show ultimate biodegradation of DCPP, but only elimination. Although a stepwise decline in the substance concentration was observed, substantial removal (39.5%) was observed after 3 hours hinting at adsorption processes. Nevertheless, the observed elimination of DCPP in the sludge samples within 28 days confirms the removal of DCPP, although the elimination is slower than in the water samples.
Remarks	As no DOC or COD was measured for the test conducted with the test item the test is not able to show ultimate biodegradation of DCPP.

Table A7_1_1_2_2-1: Inoculum

Criteria	Details
Nature	Activated sludge
Species	Mixture of polyvalent bacteria
Source/Sampling site	Collected from a communal wastewater treatment plant (ARA Therwil) on January 26, 2000.
Pretreatment	The activated sludge was washed twice with tap water one day before the test was started. A sample of the activated sludge was taken to determine the dry weight of the Suspended Solids (SS). The pre-treated sludge was aerated until use.
Initial cell concentration	0.49 g/L suspended solids (dry weight) The bacteria concentration was obtained by adding 70 mL of the pre-treated sludge (14.2 g/L SS) per 2 litres test medium.

Table A7_1_1_2_2-2: Test system

Criteria	Details
Test apparatus	Static test at 20.3 – 21.8 °C, total test volume 2 litres, continuously stirred, diffuse daylight
Aeration	O_2 concentration during test (mg/L): > 2 Test substance: 7.3 - 8.5 Ref. substance: 7.2 - 8.4 Blank: 7.9 - 8.7
Number of culture flasks/concentration	100 µg/L of the test substance 153.6 and 153.9 mg/L DOC of the reference substance
Measuring equipment	DOC determination of reference substance: TOC/DOC Analyser SHIMADZU TOC 500 DCPP and possible transformation products determined by GC/MSD

Table A7_1_1_2_2-3: Test conditions

Criteria	Details
Composition of mineral medium	For each stock solution, the mineral salts were dissolved in 1 L water (distilled water, previously checked for purity by DOC analysis) and made up to 1 L. Stock solution A: 0.5 g NH_4Cl, 33.4 g Na_2HPO_4 x 2 H_2O, 8.5 g KH_2PO_4, 21.75 g K_2HPO_4 Stock solution B: 36.4 g $CaCl_2$ x 2 H_2O Stock solution C: 22.5 g $MgSO_4$ x 7 H_2O Stock solution D: 0.25 g $FeCl_3$ x 6 H_2O 10 mL of solution A and 1 mL of solutions B, C, D were per 1 L distilled water to form the test medium.
Additional substrate	No
Suspended solids concentration	Sludge dry weight: 0.49 g/L suspended solids (SS). The bacteria concentration was obtained by adding 70 mL of the pre-treated sludge (14.2 g/L SS) per 2 litres test medium
Aeration of dilution water	See above
pH	7.0 – 8.0 Test substance: 7.0 – 7.4 Ref. substance: 7.1 – 7.8 Blank: 7.0 – 7.3
Test temperature	20 – 25 °C Test substance: 20.3 – 21.7 °C Ref. substance: 20.5 – 21.8 °C Blank: 20.5 – 21.7 °C
Other relevant criteria	None

Table A7_1_1_2_2-4: GC/MSD parameters

<table>
<tr><th>Parameter</th><th>Details</th></tr>
<tr><td>Sample volume</td><td>• 1 µL</td></tr>
<tr><td>Injector</td><td>• Split/splitless; splitless mode selected
• 250 °C
• 1 min</td></tr>
<tr><td>Column</td><td>• DB5: 30 m, 0.3 mm in diameter, 0.25 µm film
• Carrier gas: helium
• Temperature:
o 60 °C for 1 min after injection
o 180 °C (rate: 5 °C/min)
o 280 °C (rate: 15 °C/min), held for 10 min
o 260 °C at transfer line to MSD</td></tr>
<tr><td>Time/fragment windows</td><td>
<table>
<tr><th>Group no.</th><th>Time window (min)</th><th>Target substance(s)</th><th>Retention time (min, approx..)</th><th>Ions (m/z)</th></tr>
<tr><td>1</td><td>8-12</td><td>4-Cl-methoxybenzene</td><td>10.5</td><td>142, 144</td></tr>
<tr><td>2</td><td>12-20</td><td>Trimethylsilylethers of p-, m-, and o-Cl-phenols</td><td>13.3, 13.7, 14.2</td><td>185, 187</td></tr>
<tr><td>3</td><td>20-30.5</td><td>4,4’-dichloro-2’-methoxydiphenylether</td><td>29.8</td><td>268, 270</td></tr>
<tr><td>3</td><td>20-30.5</td><td>2’-trimethylsilylether of 4,4’-dichlorodiphenylether</td><td>30.0</td><td>311, 313</td></tr>
<tr><td>3</td><td>20-30.5</td><td>PCB 52 (injection standard)</td><td>29.5</td><td>290, 292</td></tr>
<tr><td>4</td><td>30.5-end</td><td>2’-trimethylsilylether of 2,4,4’-trichlorodiphenylether</td><td>31.2</td><td>351, 353</td></tr>
</table>
</td></tr>
</table>

Table A7_1_1_2_2-5: GC/MSD: recovery rates and detection limits

Recovery rates

Sample type	Range of recovery rates		
	Vessels 1, 4, and 5 ± standard deviation	Min. value	Max. value
Water	82 ± 20	59	94*
Sludge	64 ± 15	33	92

* One recovery of the internal standard was 161%, this value is not reliable and therefore not shown.

Detection limit

Compound	Detection limit (D.L.; ng)	Data significant above (ng)	D.L. derived from
Diclosan	9	18	Measurement blank
Methoxy-diclosan	5	10	Lowest measurable concentration
o-Chlorophenol	5	10	Measurement blank
m-Chlorophenol	5	10	Measurement blank/estimation
p-Chlorophenol	5	10	Measurement blank/estimation
p-Chloroanisol	44	88	Measurement blank

Table A7_1_1_2_2-6: Summary of chemical analysis (absolute content) for water and sludge samples of blank and test vessels (4 & 5; 2- and 3-chlorophenol were below the detection limit in all samples) Remark: Values in italic type are not significant.

Sample		Sample time	Sample weight (g)	Absolute content of compounds (ng)			
				Diclosan	Me-O-Diclosan	4-Chloro-anisole	4-chloro-phenol
Water samples	Blank	3 h	17.95	< d.l.	< d.l.	*25.7*	< d.l.
		1 d	23.06	*7*	< d.l.	< d.l.	< d.l.
		5 d	24.18	*7*	< d.l.	*20*	*2*
		7 d	23.81	*5*	< d.l.	*21*	*4*
		14 d	24.14	36	< d.l.	*32*	*7*
		21 d	24.01	*4*	< d.l.	< d.l.	*4*
		27 d	24.08	*8*	< d.l.	*30*	*5*
		28 d	23.54	*3*	< d.l.	*31*	*9*
	Vessel 4	3 h	17.30	1217	< d.l.	*43*	*4*
		1 d	18.70	603	*6*	*24*	*3*
		5 d	23.88	54	14	< d.l.	*2*
		7 d	23.61	31	20	*19*	*5*
		14 d	23.62	*4*	*10*	*28*	*3*
		21 d	23.92	*17*	*7*	*35*	< d.l.
		27 d	24.02	*9*	*7*	*47*	*7*
		28 d	23.74	*7*	*7*	*55*	*6*
	Vessel 5	3 h	23.23	1175	< d.l.	*28*	< d.l.
		1 d	22.24	558	*10*	97	< d.l.
		5 d	23.98	75	16	97	< d.l.
		7 d	23.93	40	29	*26*	< d.l.
		14 d	23.84	23	15	*60*	< d.l.
		21 d*	23.88	*4*	*4*	*10*	< d.l.
		27 d	24.02	*12*	13	*13*	< d.l.
		28 d	23.52	*5*	*5*	*29*	< d.l.
		*Recovery of ^{13}C6-TCS was too high (161%), therefore the data are not reliable.					
Sludge samples	Blank	3 h	5.85	78	< d.l.	*24*	< d.l.
		1 d	6.91	72	< d.l.	<= 33	<= 12
		5 d	5.51	32	< d.l.	*24*	< d.l.
		7 d	5.54	22	< d.l.	*27*	*9*
		14 d	5.11	24	< d.l.	*35*	*3*
		21 d	5.44	32	< d.l.	*35*	< d.l.
		27 d	5.30	*12*	< d.l.	*47*	< d.l.
		28 d	5.63	*14*	< d.l.	< d.l.	< d.l.
	Vessel 4	3 h	6.29	1870	*9*	*67*	< d.l.
		1 d	7.73	1025	44	*34*	< d.l.
		5 d	5.11	200	51	*31*	11
		7 d	5.84	122	88	*58*	14
		14 d	5.69	43	46	*87*	29
		21 d	5.37	31	32	< d.l.	< d.l.
		27 d	5.35	*18*	24	<= 91	< d.l.
		28 d	5.44	20	12	*40*	< d.l.
	Vessel 5	3 h	5.90	1642	15	*38*	11
		1 d	7.14	812	20	*29*	21
		5 d	5.56	113	64	*47*	< d.l.
		7 d	5.50	76	66	< d.l.	< d.l.
		14 d	5.82	43	69	< d.l.	< d.l.
		21 d	5.28	24	38	*50*	< d.l.
		27 d	5.39	21	26	< d.l.	< d.l.
		28 d	5.56	19	*10*	n.a.	< d.l.

Table A7_1_1_2_2-7: Summary of chemical analysis (concentrations) for water and sludge of blank and test vessels (4 & 5; 2- and 3-chlorophenol were below the detection limit in all samples)

Sample		Sample time	Concentration of compounds (water: µg/L; sludge: mg/kg dw)			
			Diclosan	Me-O-Diclosan	4-Chloro-anisole	4-chloro-phenol
Water	Blank	3 h	< d.l.	< d.l.	*1*	< d.l.
		1 d	*0.30*	< d.l.	< d.l.	< d.l.
		5 d	*0.29*	< d.l.	*0.83*	*0.08*
		7 d	*0.21*	< d.l.	*0.88*	*0.17*
		14 d	1.49	< d.l.	*1.33*	*0.29*
		21 d	*0.17*	< d.l.	< d.l.	*0.17*
		27 d	*0.33*	< d.l.	*1.25*	*0.21*
		28 d	*0.13*	< d.l.	*1.32*	*0.38*
	Vessel 4	3 h	70.35	< d.l.	*2.49*	*0.23*
		1 d	32.25	*0.32*	*1.28*	*0.16*
		5 d	2.26	0.59	< d.l.	*0.08*
		7 d	1.31	0.85	*0.80*	*0.21*
		14 d	*0.17*	0.42	*1.19*	*0.13*
		21 d	*0.71*	*0.29*	*1.46*	< d.l.
		27 d	*0.37*	*0.29*	*1.96*	*0.29*
		28 d	*0.29*	*0.29*	*2.32*	*0.25*
	Vessel 5	3 h	50.58	< d.l.	*1.21*	< d.l.
		1 d	25.09	*0.45*	4.36	< d.l.
		5 d	3.13	0.67	4.05	< d.l.
		7 d	1.67	1.21	*1.09*	< d.l.
		14 d	0.96	0.63	*2.52*	< d.l.
		21 d*	*0.17*	*0.17*	*0.42*	< d.l.
		27 d	*0.50*	0.54	*0.54*	< d.l.
		28 d	*0.21*	*0.21*	*1.23*	< d.l.
		*Recovery of ^{13}C6-TCS was too high (161%), therefore the data are not reliable.				
Sludge	Blank	3 h	6.7	< d.l.	*2.1*	< d.l.
		1 d	4.9	< d.l.	*<= 2.2*	<= 0.8
		5 d	2.2	< d.l.	*1.6*	< d.l.
		7 d	1.5	< d.l.	*1.9*	*0.6*
		14 d	1.7	< d.l.	*2.4*	*0.2*
		21 d	2.2	< d.l.	*2.4*	< d.l.
		27 d	*0.8*	< d.l.	*3.3*	< d.l.
		28 d	*1.0*	< d.l.	< d.l.	< d.l.
	Vessel 4	3 h	161.8	*0.8*	*5.8*	< d.l.
		1 d	79.1	3.4	*2.6*	< d.l.
		5 d	14.1	3.6	*2.2*	0.8
		7 d	8.5	6.1	*4.0*	1.0
		14 d	3.0	3.2	*6.1*	2.0
		21 d	2.2	2.2	< d.l.	< d.l.
		27 d	*1.3*	1.7	<= 6.3	< d.l.
		28 d	*1.4*	0.8	*2.8*	< d.l.
	Vessel 5	3 h	115.0	1.1	*2.7*	0.8
		1 d	56.4	1.4	*2.0*	1.5
		5 d	7.8	4.4	*3.2*	< d.l.
		7 d	5.3	4.6	< d.l.	< d.l.
		14 d	3.0	4.7	< d.l.	< d.l.
		21 d	1.7	2.7	*3.5*	< d.l.

		27 d	1.5	1.8	< d.l.	< d.l.
		28 d	1.3	*0.7*	n.a.	< d.l.

Table A7_1_1_2_2-8: Degradation of the DCPP and reference substance

Time	DCPP*		Reference substance*		
	DCPP concentration [µg/L]	Degradation [%]	DOC concentration [mg/L]		Biodegradation [%]
			Ref. mean	Blank	Ref. mean
Start	100	0	153.6 (A) 153.9 (B)	-	-
3 hours	70.35 (A) 50.58 (B)	29.65 (A) 49.42 (B)	154.6 (A) 157.3 (B)	3.7	-
1 day	32.25 (A) 25.09 (B)	67.75 (A) 74.91 (B)	151.0 (A) 152.6 (B)	3.7	2 (A) 3 (B)
5 days	2.26 (A) 3.13 (B)	97.74 (A) 96.87 (B)	100.5 (A) 100.3 (B)	4	36 (A) 37 (B)
7 days	1.31 (A) 1.67 (B)	98.69 (A) 98.33 (B)	2.1 (A) 1.7 (B)	3	100
8 days	Not reported		4.1 (A) 2.7 (B)	3.3	99 (A) 100 (B)
14 days	- (A) 0.96 (B)	> 99 (A) 99.04 (B)	1.5 (A) 2.4 (B)	3.1	100
21 days	-	> 99	3.2 (A) 2.2 (B)	3.5	100
27 days	-	> 99	2.5 (A) 2.1 (B)	4.5	100
28 days	-	> 99	2.3 (A) 2.2 (B)	4.5	100

*two tests (A + B) running in duplicate

Table A7_1_1_2_2-9: Pass levels and validity criteria for inherent biodegradation tests

	fulfilled	not fulfilled
Pass levels		
20% removal (DOC or COD)	Yes (for the removal of DCPP determined by GC/MSD)[1)]	
Pass values reached within 10-d window (within 28-d test period)	Yes (for the removal of DCPP determined by GC/MSD)[1)*)]	
Removal of reference substance (DOC or COD) > 70% within 14 d	**Yes**	
Criteria for validity		
Percentage of DOC/COD-removal of reference compound ≥ 70% within 14 days (OECD 302 B)	**Yes**	
Percentage of DOC-removal of reference compound ≥ 40% within 7 days and ≥ 65% within 14 days Average residual amount of test compound in blank tests ≥ 40% (OECD 302 C)	**Yes**	
Removal curve of DOC or COD in the test suspension indicative for biodegradation (gradual elimination over days/weeks)	Yes (for the removal of DCPP determined by GC/MSD)[1)]	

[1)] Test parameter was the dissipation of DCPP (primary degradation) and not the formation of CO_2 (ultimate degradation)
*)10-day value not available; however the pass level was reached before 10 days of incubation

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation (02)

Annex Point IIIA, XII.2.1

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (1992): Assessing the removal of the test substance during secondary wastewater treatment: D1063.01 [redacted] Report No. [redacted] unpublished, Date: 1992-08-01
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	None
2.2	**GLP**	Yes
2.3	**Deviations**	Not applicable

3 MATERIALS AND METHODS

3.1	**Test material**	Non-radiolabelled test substance [redacted] (triclosan) and radiolabelled test substance [redacted] (radiolabelled triclosan)
3.1.1	Lot/Batch number	Not reported
3.1.2	Specification	[redacted] 12.7 µCi/mg
3.1.3	Purity	[redacted]1: 99.7% [redacted] approx. 98%
3.1.4	Further relevant properties	Not relevant
3.2	**Reference substance**	Not relevant
3.2.1	Initial concentration of reference substance	Not relevant
3.3	**Testing procedure**	
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_1_1-1.
3.3.2	Test system	The test system consists of two CAS (continuous activated sludge) units. Each system is composed of a small mixing chamber which served to mix the incoming wastewater and test feed solution before they entered the aeration basin. The aeration basin contained six litres of activated sludge and was aerated by two gas dispersion tubes. The aeration basin discharged to a two liter cylindrical clarifier which was stirred by a shaft mixer at a speed of approx. 2 rpm. The clarifier has a recycle discharge in the bottom and a side tube for effluent discharge. The details are described in Table A7_1_2_1_1-2.

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation (02)

Annex Point IIIA, XII.2.1

3.3.3	Test conditions	The test conditions are described in Table A7_1_2_1_1-3.
3.3.4	Method of preparation of test solution	The primary stock solutions with D1063.02R (radio-labelled triclosan) were prepared at a radiochemical concentration of 85 µCi/mL. The test substance was weighted into a tared beaker. Approx. 50% of the intended deionised water was added. While mixing, the contents of beaker were adjusted to pH 11.0 with 1.0 N NaOH. The solution was diluted to volume and stored in the dark under refrigeration. The radioactivity level of each primary stock solution was confirmed by LSC counting in 3A toluene scintillation cocktail. The test feed solutions of the combined unlabelled and labelled triclosan prepared to contain 0.005 µCi/mL and a total of 11.1 times the nominal influent concentrations. The concentration of labelled test substance remained constant at 394 µg/L. Adjustments for the different concentrations were made using the unlabelled test substance.
3.3.5	Initial TS concentration	Nominal test substance concentrations: 40 µg/L (phase 1), 100 µg/L (phase 2), 200 µg/L (phase 3), 500 µg/L (phase 4), 1000 µg/L (phase 5), 2000 µg/L (phase 6). The level of radioactivity in the influent remained constant at approx. 1000 dpm/mL. According to the dose levels, the study was divided into six phases during which the concentration of the test substance was incrementally increased from 40 µg/L to 2000 µg/L. Phase without acclimation of the solids and high dosing level experiment (phase 7): 35 µg/L (only labelled test substance) and 750 µg/L.
3.3.6	Duration of the test	22 October 1990 until 22 March 1991 (approx. 121 days)
3.3.7	Testing procedure	Incremental phase testing: 1. Set up of the CAS units 2. Stabilisation period (11 days): both units received wastewater and deionised water without test substance 3. Acclimation period for each test substance concentration until removal of the test substance equilibrated 4. Removal period (5 days) for each test substance concentration following the acclimation period Unacclimated solids and high level dosing: 1. Switch of the solids (aeration chamber and clarifier solids) from test (unit 5) and control (unit 6) unit 2. Dosing of the test substance at 35 µg/L to unit 5 3. Unit 5 was subsequently dosed at 750 µg/L

3.3.8 Analytical methods

During the acclimation and removal period, influent, aeration mixed liquor and effluent samples were collected and assayed for ^{14}C.

Procedures of ^{14}C measurement during the acclimation period:

1. Triplicate 1 mL aliquots of influent samples (mixing chamber) were placed in 3A toluene scintillation cocktail and counted.
2. Effluent samples were taken by diverting the clarifier effluent tube into a beaker. Triplicate 5 mL aliquots from the effluent samples were counted in 5 mL deionised water and 10 mL Triton X scintillation cocktail.
3. Triplicate 5 mL aliquots from the effluent samples were acidified, incubated overnight at room temperature and counted in 5 mL deionised water and 10 mL Triton X scintillation cocktail.
4. Mixed liquor samples were taken by dipping a 2 oz. glass french square bottle into the aeration beaker. Triplicate 2 mL aliquots from the aeration basin sample were acidified, incubated overnight at room temperature, and counted in 8 mL deionized water and 10 mL Triton X scintillation cocktail.

Procedures of ^{14}C measurement during the acclimation period:

1. Triplicate 1 mL aliquots of the influent sample were placed in 3A toluene scintillation cocktail and counted.
2. 2. Triplicate 2 mL aliquots of the aeration basin were counted in 8 mL deionised water and 10 mL Triton X scintillation cocktail.
3. Triplicate 2 mL aliquots of the aeration basin sample were acidified, incubated overnight at room temperature and counted in 8 mL deionised water and 10 mL Triton X scintillation cocktail.
4. Triplicate 2 mL aliquots from the aeration basin sample were filtered through 0.4 µm Nucleopore filters. The filters were rinsed with 2 mL deionised water and counted in 10 mL deionised water and 10 mL of Triton X scintillation cocktail. The filters were not pre-soaked in unlabelled test substance prior to use.
5. Triplicate 5 mL aliquots from the effluent samples were counted in 5 mL deionised water and 10 mL Triton X scintillation cocktail.
6. Triplicate 5 mL aliquots from the effluent samples were acidified, incubated overnight at room temperature and counted in 5 mL deionised water and 10 mL Triton X scintillation cocktail.
7. Triplicate 5 mL aliquots from the effluent samples were filtered through 0.4 µm Nucleopore filters. The filters were rinsed with 5 mL deionised water and counted in 10 mL Triton X scintillation cocktail. The filtrates were acidified and counted in 10 mL Triton X scintillation cocktail.

All samples were assayed by liquid scintillation Tracor Analytic-Beta Trac 6895 according to an internal SOP.

3.3.8	Analytical methods (cont.)	Influent and effluent samples of the removal period were furthermore analyzed for parent test substance and metabolites using HPLC. In addition, the level of radioactive parent and biomass associated with the solids was determined. Procedure of sampling: Once during the 5-day removal period, overnight composite samples from both the control and the test unit were collected. The samples were covered to avoid exposure to light and iced during collection. A 500 mL aliquot of the composite from the test unit was placed in an amber bottle and acidified with 1% concentrated hydrochloric acid (HCl). In addition, 150 mL influent and 200 mL aeration mixed liquor grab samples were colleted and treated in the same manner. The samples were shipped to the sponsor and analysed there. Procedure of analysis: 1. Triplicate subsamples of in- and effluents were placed into scintillation vials with 17 mL of 3A-Toluene cocktail and assayed for radioactivity by LSC. A portion of the remaining samples were extracted twice with an equal volume of ethyl acetate in a separate funnel. Radioactivity in the combined ethyl acetate extracts was estimated by LSC. The remaining extract was evaporated at 50°C under vacuum, transferred to test tubes with ethyl acetate, dried under nitrogen and stored for chromatographic analysis. 2. Subsamples (1 mL) of mixed liquor were counted in 3A cocktail. Samples were centrifuged, and radioactivity in the supernatant determined by LSC. The supernatant was discharged and the remaining solids were sequentially extracted with acetone at 60°C for >6 h to recover the test substance and metabolites, cold trichloroacetic acid (TCA) to recover carbohydrates, ethanol/ether to recover lipids, hot TCA to recover nucleic acids, and 10N NaOH to recover protein. The radioactivity in an aliquot of each extract was determined by LSC. 3. Rad-HPLC analysis: Acetone extracts of sludge solids and ethyl extracts of in- and effluents were reconsituted in 200-300 µL acetonitrile and analysed. System: Waters 600E Detector: Waters UV 484 and Radiomatic Flo/One Beta radioactivity detector in tandem Column: Waters Radial-Pak Resolve C18 (8 x 100 mm; 5 µm) Precolumn: Waters Gard-Pak (Resolve C8) Mobile phase: Acetonitrile:water (52:48) at al flow rate of 2 mL/min Scintillation cocktail: FloScint III, flow rate 8 mL/min 4. Rad-TLC Analysis: Subsamples of selected extracts were spotted onto Silica Gel 60 HPTLC plates and developed with chloroform as the mobile phase. The plates were dried and scanned using a Radiomatic TLC Scanner to localize and quantify the radioactive peaks.
3.3.9	Sampling	Acclimation period: Collection of samples three times a week. Removal period: Influent, aeration mixed liquor and effluent samples were removed once a day and radioassayed.
3.3.10	Intermediates/	See point 3.3.8

	degradation products	
3.3.11	Monitoring parameter during the test	COD (chemical oxygen demand), TSS (total suspended solids), BOD (biological oxygen demand), pH, ammonia (NH_3), nitrate nitrogen (NO_3), sludge volume index (SVI), pumping flow rates, sludge blanket height, and dissolved oxygen
3.3.12	Controls	Two CAS units were used in this study, on of them serving as a control (without test substance)
3.3.13	Statistics	Not relevant

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Degradation	The composition of radioactivity in CAS influents and effluents and the removal of the test substance as a function of test substance influent concentration are summarized in Table A7_1_2_1_1-4. Table A7_1_2_1_1-5 shows the radioactivity associated with sludge solids that were the test substance or that had been incorporated into biomass. Table A7_1_2_1_1-6 summarised the removal of Triclosan in the CAS unit dependent on various dissipation mechanisms. Tables A7_1_2_1_1-7 and A7_1_2_1_1-8 refer to the dissipation of the test substance in the CAS unit following shock loading to an acclimated unit.
4.1.2	Graph	The results are presented in tabular form (Tables A7_1_2_1_1-4 to A7_1_2_1_1-8
4.1.3	Other observations	One day after the start of the acclimation period of phase 1 (40 µg/L), the test feed solution was assayed for ^{14}C as it entered the mixing chamber. It was determined that a loss of approx. 70% of the test substance was occurring between the test feed solution bottle and the mixing vessel. The loss was identified to occur in the delivery tubing. An additional experiment was conducted to determine adsorption of the test substance to various types of tubing. Based on these results the delivery tubing was changed from tygon to teflon tubing. The Masterflex tubing was changed from C-flex tubing to a tygon tubing specifically formulated for use with hydrocarbons.
4.1.4	Degradation of reference substance	The degradation of a reference substance has not been determined
4.1.5	Intermediates/ degradation products	Intermediates/degradation products have not been specifically identified. In the ethyl acetate extracts of the effluents polar breakdown products could be observed, however, their formation was considered to be transient (see Point 5.2).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A continuous activated sludge study (CAS) was conducted in order to assess the treatability of [redacted] (triclosan) during secondary wastewater treatment. The study was divided into six phases, during which the test concentrations were incrementally increased from 40 µg/L to 2000 µg/L. A seventh phase consisted of a testing period using unacclimated solids and a high level dosing experiment. For each test concentration (phases 1 to 6) the CAS units were allowed to stabilize for 11 days. Afterwards an acclimation period was conduced, lasting until removal of the test substance equilibrated. Subsequently a 5 day removal period followed up. During the acclimation and the removal periods, influent, effluent and aeration mixed liquor samples were taken and assayed for ^{14}C. Furthermore, in- and effluent samples were partly analysed for parent

substance and metabolites. The level of radioactivity associated with the solids was determined.

5.2 Results and discussion

Table A7_1_2_1_1-4 shows the percentage of the total radioactivity in the influents and effluents of the removal phase, which extracted into ethyl acetate as well as the percentage of the total radioactivity that was identified as Triclosan by HPLC analysis. In the case of the influents, much of the radioactivity (usually >90%) extracted into ethyl acetate and chromatographed as test substance. In contrast, the percentage of effluent radioactivity that extracted into ethyl acetate was highly variable. During phases 1 to 3, 55 to 92% of effluent radioactivity was extractable. During phases 3 to 6, the percentage extractable decreased to 8 to 37%. HPLC analyses revealed that none of this radioactivity was parent test substance. Instead, it consisted of radiolabelled materials that eluted very early in the chromatograms. This partitioning and chromatographic behaviour suggests that most of the effluent radioactivity consists of polar breakdown products. The absence of high levels of these materials indicates that their formation is transient and that they are not persistent. The only exception was the phase 1 acclimation sample, which showed > 92% of the original test substance in the effluent. However, comparison of ^{14}C-[redacted] levels in the influent and effluent indicated that removal of the parent was virtually complete at all influent concentrations tested.

Table A7_1_2_1_1-5 shows the percentage of the radioactivity associated with sludge solids, either as the parent test substance or being incorporated into biomass. Radioactivity in biomass was determined by totaling the amount of ^{14}C in various sequential extracts that selectively recover individual biomass constituents. This approach was validated by spiking ^{14}C-triclosan into abiotic sludge samples, with the result of 85.1% recovered as triclosan and 0.5% recoverd as ^{14}C biomass. During phases 1 to 6, 30 to 63% of the radioactivity recovered from the solids was parent, while 37 to 67% was in biomass components. Based upon these analyses, 5 to 12% of the test substance radioactivity was removed by sorption and 75 to 88% was removed by conversion to CO_2 or biomass under steady state conditions (cf. Table A7_1_2_1_1-6).

During phase 7, > 80% of the radioactivity in the influents partitioned and chromatographed as triclosan but only 11 to 29% of the radioactivity in the effluents was parent (Table A7_1_2_1_1-7). Comparison of ^{14}C-triclosan levels in the in- and effluent indicated that specific removal of the parent during this phase was 94 to 98% and did not significantly change in response to high level shock loading. During and following the shock loading, the ratio of test substance to biomass on the solids was somewhat higher than that observed during the former phases, suggesting that removal by sorption increased in prominence during the shock load (Table A7_1_2_1_1-8). The shock loadings of the test substance did not have an adverse effect on the CAS system performance.

5.3 Conclusion

Characterisation of the radioactivity in the influents and effluents indicate that the parent compound triclosan is effectively removed at concentrations ranging from 40 to 2000 µg/L, and under the conditions of shock loading. Analysis of the radioactivity associated with solids indicates that a large fraction of the ^{14}C is present in the form of biomass. Thus, during this test simulating activated sludge treatment, 75 – 88% of the parent substance was removed by conversion to CO_2 or biomass.

5.3.1 Reliability

1-2

5.3.2 Deficiencies

The degradation products in the effluents have neither been identified nor quantified; the sludge was not combusted; a real mass balance was not established

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	May 2012
Materials and Methods	Applicant's version is acceptable.
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	2
Acceptability	Acceptable for environmental risk assessment.
Remarks	Triclosan is removed in the continuous activated sludge system, which simulates a wastewater treatment plant, at 75 – 88% of the parent substance by conversion to CO_2 or biomass. It was observed that in phase I (the lowest used concentration) > 92% of the active substance was recovered from effluent in the acclimatisation period. This illustrates that adaptation is a critical factor on degradation rate and extent.

Table A7_1_2_1_1-1: Description of Inoculum

Criteria	Details
Nature	1. Activated sludge for setting up the CAS unit 2. Wastewater from the same treatment facility
Species	Mixed population of aquatic microorganisms
Source/Sampling site	Local municipal wastewater treatment facility (Avondale Sewage Treatment Plant, Avondale, Pennsylvania), receiving primarily domestic wastewater.
Preparation of inoculum for exposure	The sludge was screened to remove large clumps. The total suspended solids level was determined and based on this the sludge was distributed to the CAS units at a concentration of approx. 2500 mg/L. Wastewater was collected two times a week , filtered through a 20 mesh stainless steel screen and stored under refrigeration in fifty gallon drums. Each drum was equipped with a mechanical mixer to keep suspended solids uniformly distributed
Pretreatment	None
Initial cell concentration	Not reported

Table A7_1_2_1_1-2: Description of the test system

Criteria	Details
Mixing chamber:	The test substance and wastewater was added to the aeration basin via the mixing chamber. The test substance feed solution was held in a glass container (wrapped with foil) under refrigeration. Both, the test substance and wastewater were pumped via a peristaltic pump into the mixing chamber at a target flow rate of 1.5 mL/min (test substance) and 15.2 mL/min (wastewater). From the mixing chamber, wastewater entered the aeration basin via an overflow tube.
Aeration basin	The aeration basin contained six litres of activated sludge and was aerated by two gas dispersion tubes using compressed air. The target inflow rate of (spiked) wastewater was 1,000 mL/h (910 mL wastewater and 90 mL feed solution) in a hydraulic residence time (HRT) in aeration of 6.0 ± 0.5 h. The solids retention time was maintained between 4 – 12 days. The test was run with two aeration units, one of them serving as control (without test substance).
Clarifiers	The aeration basins discharged to individual 2-L cylindrical clarifiers which were stirred by a shaft mixer at a speed of approx. 2 rpm. The clarifiers had a recycle discharge in the bottom and a side tube for effluent discharge.
Aeration device	Two gas dispersion tubes using compressed air
Test performed in closed vessels due to significant volatility of TS	Not relevant

Table A7_1_2_1_1-3: Description of the test conditions

Criteria	Details
Composition of medium	Six litres of mixed sludge wastewater in aeration. The test medium was gained from the local municipal wastewater treatment facility, receiving primarily domestic wastewater.
Additional substrate	None
Test temperature	20.7 – 23.5°C
pH	Control unit 6: influent: 7.0 – 8.6 effluent: 7.1 – 8.0 Test unit 5: influent: 7.0 – 8.4 effluent: 7.1 – 8.0
Aeration of aeration basin	Via two gas dispersion tubes using compressed air
Suspended solids concentration	The total suspended solids concentration was measured regularly.
Concentration of inoculum	Not relevant
Other relevant criteria	None

Table A7_1_2_1_1-4: Composition of radioactivity in CAS influents and effluents and removal of triclosan as a function of the test substance influent concentration

Sampling	^{14}C recovered in ethyl acetate (%)	^{14}C identified as triclosan (%)	Removal of triclosan (%)	Overall removal (%)
Phase 1 (40 µg/L) R[1)]				
Influent	98.6	98.6		
Effluent	54.5	ND	> 99.5	90.3
Phase 1 (40 µg/L) A[2)]				
Effluent	92.1	92.1	NC	60.1
Phase 2 (100 µg/L) R				
Influent	90.5	90.5		
Effluent	54.7	ND	> 99.5	86.9
Phase 3 (200 µg/L) R				
Influent	91.3	91.3		
Effluent	63.2	ND	> 99.5	93.8
Phase 3 (200 µg/L) A				
Influent	100.0	100.0		
Effluent	32.6	ND	> 99.5	94.5
Phase 4 (500 µg/L) R				
Influent	77.6	77.6		
Effluent	29.2	ND	> 99.5	85.8
Phase 5 (1000 µg/L) R				
Influent	100.0	100.0		
Effluent	31.6	ND	> 99.5	93.2
Phase 6 (2000 µg/L) R				
Influent	100.0	100.0		
Effluent	8.4	ND	> 99.5	95.3

ND = Not detected (< 4 dpm test substance/mL); NC = not calculated (no influent sample)
[1)] R = Removal period;
[2)] A = Acclimation period

Table A7_1_2_1_1-5: Composition of radioactivity associated with CAS solids as a function of triclosan influent concentration during the removal period

Sampling	Recovered as ^{14}C-triclosan (%)	Recovered as ^{14}C-biomass (%)	Total recovery (%)
D1063 spiked abiotic sludge	85.1	0.5	85.6
Phase 1 (40 µg/L)	50.0	37.0	87.0
Phase 2 (100 µg/L)	63.1	48.8	111.9
Phase 3 (200 µg/L)	30.0	45.1	75.1
Phase 3 (200 µg/L)[1)]	32.9	51.4	84.3
Phase 4 (500 µg/L)	47.8	52.7	100.5
Phase 5 (1000 µg/L)	33.5	51.6	85.1
Phase 6 (2000 µg/L)	39.2	66.9	106.1

[1)] Acclimation period

Table A7_1_2_1_1-6: Dissipation of triclosan during the removal phase: mean overall removal, removal by biodegradation, and removal by sorption

Phase	Concentration (µg/L)	Removal range (%)	Mean over-all removal (%)	Parent removal (%)	Biodegradation[1)] (%)	Sorption (%)
1	40	75.5 – 89.8	83.8 ± 5.9	> 99.5	74.7	9.1
2	100	85.6 – 90.3	88.0 ± 1.9	> 99.5	75.5	12.5
3	200	91.0 – 94.3	92.7 ± 1.5	> 99.5	85.7	7.0
4	500	85.2 – 87.2	86.1 ± 0.9	> 99.5	76.3	9.8
5	1000	91.5 – 94.7	93.2 ± 1.2	> 99.5	87.8	5.4
6	2000	93.8 – 96.4	95.4 ± 1.0	> 99.5	87.1	8.3

[1)] Biodegradation = Mineralized or incorporated into biomass

Table A7_1_2_1_1-7: Composition of radioactivity in CAS influents and effluents and removal of triclosan following shock loading of 750 µg/L to a unit acclimated to 35 µg/L

Sampling	^{14}C recovered in ethyl acetate (%)	^{14}C identified as triclosan (%)	Removal of triclosan (%)	Overall removal (%)
Shock loading 1 (3/13/91)				
Influent	82.7	82.7		
Effluents				
1 h	35.4	18.4	96.7	86.1
3 h	38.9	24.3	95.7	85.6
8 h	31.0	17.2	97.1	82.9
Overnight composite	43.4	28.7	94.0	82.5
Before second shock (3/15/91)				
Influent	88.5	88.5		
Effluent	44.2	21.2	97.0	85.3
Shock loading 2 (3/15/91)				
Influent	87.4	87.4		
Effluents				
3 h	45.0	11.0	98.3	86.8
Overnight composite	55.2	28.1	94.9	81.5

Table A7_1_2_1_1-8: Composition of radioactivity associated with CAS solids prior to and following shock loadings of 750 µg/L to a unit acclimated to 35 µg/L (phase 7)

Sampling	Recovered as ^{14}C-triclosan (%)	Recovered as ^{14}C biomass (%)	Total recovery (%)
Test substance spiked with abiotic sludge	85.1	0.5	85.6
Shock loading 1 (3/13/91) 10 h 24 h	 54.7 57.0	 ND 32.1	 ND 89.1
Before second shock (3/15/91)	60.2	35.2	95.4
Shock loading 2 (3/15/91) 10 h 24 h	 74.0 40.5	 34.7 28.3	 108.7 68.8

ND = Not done (sample was lost)

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation (03)

Annex Point IIIA, XII.2.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1998): Assessing the removal of the test substance during secondary wastewater treatment: [redacted] Report No [redacted] unpublished, Date: 1998-03-16	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	None	
2.2	**GLP**	Yes	
2.3	**Deviations**	Not applicable	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance [redacted](triclosan) and radiolabelled test substance [redacted] (radiolabelled triclosan)	
3.1.1	Lot/Batch number	Radiolabelled triclosan: [redacted] non-radiolabelled triclosan: not reported	
3.1.2	Specification	Radiolabelled triclosan: 140.4 µCi/mg	x
3.1.3	Purity	Non-radiolabelled triclosan: 99.8% Radiolabelled triclosan: 97.6% and 98.6% (two shipments)	
3.1.4	Further relevant properties	Not relevant	
3.2	**Reference substance**	Not relevant	
3.2.1	Initial concentration of reference substance	Not relevant	
3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The inoculum is described in Table A7_1_2_1_1-1.	
3.3.2	Test system	The test system consists of five CAS units (continuous activated sludge). Unit 1 was designated the control, units 2 to 5 were dosed at different concentrations. Each system is composed of a small mixing chamber which served to mix the incoming wastewater and test feed solution before they entered the aeration basin. The aeration basin contained six litres of activated sludge and was aerated by gas dispersion tubes. The aeration basin discharged to a two liter cylindrical clarifier which was stirred by a shaft mixer at a speed of approx. 2 rpm. The clarifier has a	

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation (03)

Annex Point IIIA, XII.2.1

		recycle discharge in the bottom and a side tube for effluent discharge. The details are described in Table A7_1_2_1_1-2.
3.3.3	Test conditions	The test conditions are described in Table A7_1_2_1_1-3.
3.3.4	Method of preparation of test solution	Two shipments of radiolabelled Triclosan were required to complete die study. Each aliquot was used to prepare a primary stock solution containing a target radiochemical concentration of 100 µCi/mL using the following procedure: The entire contents were transferred to a small beaker and rinsed with 2 ml deionised water, previously adjusted to a pH of 10 with 1 N NaOH. While mixing, 1.0 N NaOH was added drop by drop until no visible test substance remained. The mixture was then diluted to the appropriate volume with deionised water, transferred to a serum vial, wrapped in foil, and refrigerated when not being used. Test feed solutions were prepared using a combination of the unlabelled and radiolabelled forms of the test substance. During the stabilisation period, only the unlabelled form of die test substance was used. The radiolabelled form of the chemical was added as tracer during the equilibration and testing phases. The test feeds were prepared weekly and stored in glass containers wrapped in foil under refrigeration. The test feeds were prepared at each concentration using the following procedure: Approximately 15 liters deionised water (adjusted to pH 11.0) was added to a 5-gallon glass bottle. The required quantity of unlabelled test substance was weighed into a tared beaker and approx. 50 mL deionised water was added. While mixing, 1 N NaOH was added to the beakers until no test substance was visible (~ pH 11.0), diluted to approx. 80 mL and added to the previously prepared 5-gallon bottle by rinsing thoroughly with deionised water, followed by dilution to 17 liters. The appropriate volume of radiolabelled stock solution was added, the test feed was mixed and the pH determined.
3.3.5	Initial TS concentration	Units 2 though 5 were dosed at total nominal influent concentrations of: 7.5, 11.0, 20.0 and 50.0 µg/L. Since the background concentration was approx. 6 µg/L, the test substance was added at the following concentrations: Stabilization period (addition of non-radiolabelled triclosan, only): 1.5, 5.0, 14.0 and 44.0 µg/L. Equilibration period: radiolabelled triclosan: 1.5, 2.75, 5.0 and 12.5 µg/L plus non-radiolabelled triclosan: 0, 2.25, 9.0 and 31.5 µg/L
3.3.6	Duration of the test	Stabilisation phase: 14 days Equilibration phase: approx. 1 month Testing phase: 5 days
3.3.7	Testing procedure	1. Set up of the CAS units 2. Stabilisation period (14 days): units 2 to 5 received wastewater mixed with non-radiolabelled triclosan at target concentrations, unit 1 received wastewater and deionised water without test substance 3. Equilibration period (approx. 1 month): units 2 to 5 received wastewater mixed with radiolabelled and non-radiolabelled triclosan at target concentrations, unit 1 received wastewater and deionised water without test substance 4. Testing phase (5 days): the same as for the equilibration phase

Section A7.1.2.1.1 Biological sewage treatment: Aerobic biodegradation (03)

Annex Point IIIA, XII.2.1

3.3.8	Analytical methods	During the equilibration period, the test feed, influent, and mixed liquor samples were periodically collected from the test units and analysed by LSC using the following technique: • Triplicate 1 mL aliquots of the test feed samples were cocktailed with Ultima Gold and analyzed by LSC. • Triplicate 1 mL aliquots of the influent samples were cocktailed with Ultima Gold and analyzed by LSC. • Triplicate 1 mL aliquots of the influent samples were acidified with 1 mL 0.5% HCI and allowed to stand overnight open to the atmosphere. Following incubation the vials were cocktailed with Ultima Gold XR and analysed by LSC. • Triplicate 5 mL aliquots from the effluent samples were acidified with 1 mL 0.5 % HCl, allowed to incubate overnight, cocktailed with Ultima Gold XR, and assayed by LSC. • Triplicate 1 mL aliquots from the aeration basins were acidified with 1 ml of 0.5% HCI, allowed to incubate overnight, cocktailed with Ultima Gold XR, and assayed by LSC. • Triplicate 5 mL aliquots of each wastewater collection were assayed by LSC to determine background radioactivity information. During the testing phase, test feed, influent, mixed liquor, and effluent samples were removed at five intervals and assayed for the level of radioactivity present. • Triplicate 1 mL aliquots of the test feed samples were cocktailed in Ultima Gold and assayed by LSC. • Triplicate 1 mL aliquots of the influent samples were cocktailed in Ultima Gold and assayed by LSC. • Triplicate 1 mL aliquots of the influent samples were acidified with 1 mL 0.5% HCl, incubated overnight, cocktailed in Ultima Gold XR, and assayed by LSC. • Triplicate 1 mL aliquots of the mixed liquors were cocktailed in Ultima Gold and assayed by LSC. • Triplicate 1 mL aliquots of the mixed liquors were acidified with 1 mL of 0.5% HCl, incubated overnight, cocktailed with Ultima Gold XR, and assayed by LSC.

3.3.8 Analytical methods (cont.)

- Triplicate 2 mL aliquots of the mixed liquor were filtered through 0.4 µm Nucleopore filters. A preliminary check determined that it was not necessary to presoak the filters in test substance prior to use. Following filtration, die filters were rinsed with 2 mL deionised water, cocktailed with Ultima Gold, and assayed by LSC. The filtrates were acidified with 1 mL 0.5% HCl, incubated overnight, cocktailed with Ultima Gold XR, and assayed by LSC.
- Triplicate 5 mL aliquots from the effluent samples were cocktailed with Ultima Gold and assayed by LSC.
- Triplicate 5 mL aliquots from the effluent samples were acidified with 1 mL of 0.5% HCl, incubated overnight, cocktailed with Ultima Gold XR, and assayed by LSC.
- Triplicate 5 mL aliquots from the effluent samples were filtered through 0.4 µm Nucleopore Filters. The filters were rinsed with 5 mL deionised water, cocktailed with Ultima Gold and assayed by LSC. The filtrates were acidified with 1 mL 0.5 % HCl, incubated overnight, cocktailed with Ultima Gold XR, and assayed by LSC.

All LSC measurements were conducted with a Packard Tri-Carb 2500 Liquid Scintillation Counter according to standard operating practices.

The parent and metabolite analysis was done according the following procedures on three of the five testing days:

- Triplicate 10 mL aliquots of the mixed liquor samples were transferred to 15-mL test tubes. The samples were centrifuged. Triplicate aliquots of the centrates were assayed by LSC. The solids were stored at -80°C until lyophilized on a Virtis Benchtop Freeze Dryer.
- Triplicate 5 mL test feed and 10 mL influent aliquots were transferred to 15-mL test tubes. 100 mL aliquots of effluent were transferred to 125-mL Erlenmeyer flasks. The effluent samples were acidified with 1 % HCl to pH 2. Samples were immediately capped and flash frozen in a dry ice/acetone bath. The frozen samples were stored until lyophilized.
- On one of the five sampling days, a 2 liter aliquot of each effluent sample was passed through pure glass fiber filters. Duplicate 100 mL aliquots of the filtrates were placed into 125-mL Erlenmeyer flasks and immediately flash frozen. The frozen samples were stored until lyophilized. The filters were stored at the same temperature until they were extracted for RAD-TLC analysis.
- On one of the five sampling days, triplicate 10 mL aliquots of mixed liquor from the control unit were transferred to 15-mL test tubes using a wide-tipped pipette. Mercuric chloride was added to the mixed liquor at a concentration of 1 g/L prior to autoclaving for 60 minutes. The samples were then centrifuged and the centrate discarded. The remaining solids were spiked with the test substance at a dpm level equivalent to that recovered on the filters at each treatment. The samples were stored at -80°C until extracted for RAD-TLC analysis.

For each treatment, two of the three samples were extracted with 10 mL acetone at ~ 60°C for ~ 18 hours. Duplicate 100 µL (test feeds/influents) or 500 µL (mixed liquor/effluents) aliquots of each solvent extract were cocktailed with Ultima Gold and assayed by LSC. The remaining extracts were dried completely under nitrogen and redissolved in a known amount of the same extraction solvent. The scintillation vials used in the dry-down procedure were pretreated with an unlabeled (~5 mg/L) solution of the test substance. The third sample from each treatment remained archived in the freezer. A subsample of each

		redissolved extract was spotted onto prechanneled 60 Ä Silica Gel TLC plates (Whatman). Aliquots of the parent solution were spotted on the outer lanes. The plates were developed in chloroform, allowed to dry, and scanned using a Bioscan Imaging 200 System. To confirm the identification of parent and metabolite peaks, selected extracts were spiked with the standard solution (parent) and re-analyzed. The radioactivity in the residual mixed liquor solids, including abiotic controls, was determined by combustion using a Packard Sample Oxidizer (Model 307). Wastewater analysis: Once per day during the testing phase, a liter aliquot of wastewater was collected in a plastic coated glass amber bottle. The wastewater was preserved to pH <2 and stored at a temperature <4°C prior shipment for analyses.
3.3.9	Sampling	Equilibration period: Test feed, influent, effluent and mixed liquor samples were taken periodically and analysed by LSC Testing period: Test feed, influent, effluent and mixed liquor samples were removed at five intervals and assayed for radioactivity. Additionally, on three of five testing days the test media were analysed for parent and metabolites by RAD-TLC.
3.3.10	Intermediates/ degradation products	See points 3.3.8 and 3.3.9
3.3.11	Monitoring parameter during the test	COD (chemical oxygen demand), TSS (total suspended solids), pH, ammonia (NH_3), nitrate nitrogen (NO_3), sludge volume index (SVI), pumping flow rates, sludge blanket height, and dissolved oxygen (DO)
3.3.12	Controls	Five CAS units were used in this study, on of them serving as a control (without test substance)
3.3.13	Statistics	Not relevant

4 RESULTS

4.1	**Degradation of test substance**	
4.1.1	Degradation	The removal (%) of the total and parent radioactivity in the mixing chamber and the total CAS system is summarized in Table A7_1_2_1_1-4. Tables A7_1_2_1_1-5 to A7_1_2_1_1_8 show the distribution of radioactivity (in % of initial dose and µg/L) for each dose level.
4.1.2	Graph	The results are presented in tabular form (Tables A7_1_2_1_1-4 to A7_1_2_1_1-8).
4.1.3	Other observations	None
4.1.4	Degradation of reference substance	The degradation of a reference substance has not been determined.
4.1.5	Intermediates/ degradation products	The intermediates being either present in the effluents and or sorbed to the solids are referred to in Tables A7_1_2_1_1-4 to A7_1_2_1_1-8.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	A continuous activated sludge study (CAS) was conducted in order to assess the treatability of triclosan during secondary wastewater treatment. The study was divided into three phases: set up/stabilisation

(14 days), equilibration (1 month) and testing (5 days). Four test units were dosed at total nominal influent concentrations of 7.5, 11.0, 20.0 and 50.0 µg/L, a fifth unit served as a control (without test substance). Each test unit consisted of a small mixing chamber (mixing of wastewater and test substance feed solution), an aeration basin containing 6 L of activated sludge (aerated by gas dispersion tubes) and 2-liter cylindrical clarifier which were stirred. The clarifier had a recycle discharge in the bottom and a side tube for effluent discharge. During the equilibration and testing periods, influent, effluent and aeration mixed liquor samples were taken and assayed for ^{14}C. Furthermore, during the testing phase the test media were analysed for parent and metabolites by RAD-TLC.

5.2 Results and discussion

The test substance triclosan was removed almost completely in all CAS systems (98.2 – 99.3% removal). Also the total radioactivity removal in the CAS units was high, as indicated by a figure of > 82% in the treated CAS systems. At all dose levels, the dissipation of triclosan was mainly due to complete mineralisation (73.9% - 76.7 % of the initial radioactivity). Besides, 14.2% to 17.1% remained unextractable with the solids. Only less than 5 % of the initial radioactivity was either sorbed onto solids or present in the effluents, respectively. The distribution of the radioactivity amongst parent compound and metabolites for each dose level is as follows:

7.5 µg/L treatment (*cf.* Table 7_1_2_1_1-5):

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.8 % of the dosed radioactivity was present on the solids as parent. The other activity in the extract was divided among two unidentified polar peaks at Rfs -0.022 and 0.028 and a non-polar peak at Rf 0.751. The remaining radioactivity was associated with the solids. Combustion of extracted abiotic sludge solids spiked with parent indicated ~ 4% of the spike behaved as biomass constituents.
RAD-TLC analysis of the effluent extracts revealed ~ 1.5 % of the dosed radioactivity was present as parent. This equates to a 98.21% removal of parent equivalent to an effluent concentration of 0.11 µg/L. The remaining activity in die effluent was divided among three unidentified polar peaks and a non-polar peak at Rf 0.714.

11.0 µg/L treatment (*cf.* Table 7_1_2_1_1-6):

RAD-TLC analysis of the mixed liquor solids extracts revealed that 1.9% of the dosed radioactivity was present on the solids as parent. The other activity in the extract was divided among two unidentified polar peaks and a non-polar peak at Rf 0.653. The remaining radioactivity was associated with the sludge solids. Combustion of extracted abiotic solids spiked with parent indicated ~2.5% of die spike behaved as biomass constituents.
RAD-TLC analysis of the effluent extracts revealed 0.64% of the dosed radioactivity was present as parent in die effluent. This equates to 99.33% removal of parent equivalent to an effluent concentration of 0.07 µg/L. The remaining activity in the effluent was found in three unidentified polar peaks and in a non-polar peak at Rf 0.768.

20 µ/L treatment (*cf.* Table 7_1_2_1_1-7):

RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.9% of the dosed radioactivity was present on the solids as parent. The other activity in the extract was divided among two unidentified polar peaks and a non-polar peak at Rf 0.694. The remaining radioactivity was associated with the solids. Combustion of extracted abiotic sludge solids spiked with parent indicated ~4% of die spike behaved as biomass constituents.
RAD-TLC analysis of the effluent extracts revealed 0.90% of the dosed radioactivity was present as parent in the effluent. This equates to a 99.05% removal of parent equivalent to an effluent concentration of 0.18 µg/L. The remaining activity in the effluent was divided among three unidentified polar peaks and a non-polar peak at Rf 0.741.

5.2	**Results and discussion (cont.)**	**50.0 µg/L treatment (*cf.* Table 7_1_2_1_1-8):** RAD-TLC analysis of the mixed liquor solids extracts revealed that ~1.4% of the dosed radioactivity was present on the solids as parent. The other activity in the extract was divided among two unidentified polar peaks and a non-polar peak at Rf 0.694. The remaining radioactivity was associated with the sludge solids. Combustion of extracted abiotic solids spiked with parent indicated ~4% of the spike behaved as biomass constituents. RAD-TLC analysis of the effluent extracts revealed 0.72% of the dosed radioactivity was present as parent in the effluent. This equates to a 99.09% removal of parent equivalent to an effluent concentration of 0.36 µg/L. The remaining activity in the effluent was found in three unidentified polar peaks and in a non-polar peak at Rf 0.682.	
5.3	**Conclusion**	The test substance triclosan was removed almost completely in all CAS systems (98.2 – 99.3% removal). At all dose levels, the dissipation of triclosan was mainly due to complete mineralisation (73.9% - 76.7 % of the initial radioactivity). Besides, 14.2% to 17.1% remained unextractable with the solids. Only less than 5 % of the initial radioactivity was either sorbed onto solids or present in the effluents, respectively. Besides triclosan, mainly polar breakdown products could be identified in the effluents or sorbed onto solids. However, all breakdown products observed occurred at marginal low amounts, only (< 2.5% of the initial radioactivity for a single breakdown product).	
5.3.1	Reliability	1	
5.3.2	Deficiencies	None	x

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2012
Materials and Methods	Specification: the position of the radiolabelling is missing.
Results and discussion	Elimination curve should be included.
Conclusion	Deficiencies: Batch no. is not reported for the non-radiolabelled triclosan
Reliability	1
Acceptability	Acceptable for environmental risk assessment.
Remarks	The study guideline is comparable to OECD TG 303. The used test substance concentrations appear to be in agreement with expected values found in the literature on waste water measurements based on household and manufacturing wastewaters.

Table A7_1_2_1_1-1: Description of Inoculum

Criteria	Details
Nature	1. Activated sludge for setting up the CAS unit 2. Wastewater from the same treatment facility
Species	Mixed population of aquatic microorganisms
Source/Sampling site	Local municipal wastewater treatment facility (Downington Regional Water Pollution Control Center, DRWPCC). The sludge was collected from an aeration basin and aerated upon receipt.
Preparation of inoculum for exposure	The sludge was screened to remove large clumps. The total suspended solids level was determined (see below). Each CAS unit contained six liters of activated sludge in the aeration basin at a solids level adjusted to 2500 mg TSS/L. 3.8 liters of sludge from the DRWPCC was added to each CAS unit. The sludge was diluted to volume with wastewater from the same treatment plant. Wastewater was collected two times a week, filtered through a 20 mesh stainless steel screen and stored under refrigeration in fifty gallon drums. Each drum was equipped with a mechanical mixer to keep suspended solids uniformly distributed
Pretreatment	None
Initial total suspended solids concentration (TSS)	4040 mg/L

Table A7_1_2_1_1-2: Description of the test system

Criteria	Details
Mixing chamber:	The test substance and wastewater was added to the aeration basin via the mixing chamber. The test substance feed solution was held in a glass container (wrapped with foil) under refrigeration. Both, the test substance and wastewater were pumped via a peristaltic pump into the mixing chamber at a target flow rate of 1.5 mL/min (test substance) and 15.2 mL/min (wastewater). From the mixing chamber, wastewater entered the aeration basin via an overflow tube.
Aeration basin	The aeration basin contained six litres of activated sludge and was aerated by two gas dispersion tubes using compressed air. The target inflow rate of (spiked) wastewater was 1,000 mL/h (910 mL wastewater and 90 mL feed solution) in a hydraulic residence time (HRT) in aeration of 6.0 ± 0.5 h. The average sludge retention time (SRT) was 11 days. The test was run with five aeration units, one of them serving as control (without test substance).
Clarifiers	The aeration basins discharged to individual 2-L cylindrical clarifiers which were stirred by a shaft mixer at a speed of approx. 2 rpm. The clarifiers had a recycle discharge in the bottom and a side tube for effluent discharge.
Aeration device	Two gas dispersion tubes using compressed air
Test performed in closed vessels due to significant volatility of TS	Not relevant

Table A7_1_2_1_1-3: Description of the test conditions

Criteria	Details
Composition of medium	Six litres of mixes sludge wastewater in aeration. The test medium was gained from the local municipal wastewater treatment facility, receiving primarily domestic wastewater.
Additional substrate	None
Test temperature	Mean test temperature: 22.4 °C
pH	Wastewater: 7.1 – 7.5 Influent: 7.3 – 8.1 Mixed liquor: 6.6 – 7.3 Effluent: 7.0 – 8.0
Aeration of aeration basin	Via two gas dispersion tubes using compressed air
Initial total suspended solids concentration (TSS)	4040 mg/L
Concentration of inoculum	Not relevant
Other relevant criteria	None

Table A7_1_2_1_1-4: Removal (%) of the total and parent radioactivity in the mixing chamber and the total CAS system

Triclosan	7.5 µg/L	11.0 µg/L	20.0 µg/L	50.0 µg/L
% Removal: Total radioactivity				

Mixing chamber	-1.59 ± 6.11	-11.03 ± 4.89	-20.44 ± 6.03	-9.96 ± 4.33
Total CAS system	86.86 ± 1.23	87.55 ± 0.62	82.48 ± 2.64	88.81 ± 1.35
% Removal: Parent				
Mixing chamber	-18.20 ± 17.63	-19.89 ± 22.30	-18.36 ± 7.69	-28.14 ± 18.50
Total CAS system	98.21 ± 0.42	99.33 ± 0.49	99.05 ± 0.26	99.09 ± 0.28

Table A7_1_2_1_1-5: Distribution of radioactivity (in % of initial dose and µg/L) of the 7.5 µg/L dose level

Triclosan 7.5 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralisation ($^{14}CO_2$)[1)]	73.86 ± 2.27	Not applicable
Remaining with the solids	17.01 ± 1.89	1.28 ± 0.14
Sorbed onto solids as:		
Parent	1.78 ± 0.39	0.13 ± 0.03
Metabolite:		
-0.022	0.20 ± 0.06	0.02 ± 0.00
0.028	1.07 ± 0.25	0.08 ± 0.02
0.751	0.98 ± 0.14	0.07 ± 0.01
Total sorbed	4.04 ± 0.64	0.31 ± 0.05
Present in effluent as:		
Parent	1.45 ± 0.37	0.11 ± 0.03
Metabolite:		
-0.029	0.54 ± 0.28	0.04 ± 0.02
0.019	1.68 ± 0.35	0.13 ± 0.03
0.161	0.91 ± 0.23	0.07 ± 0.02
0.714	0.50 ± 0.07	0.04 ± 0.01
Total effluent	5.09 ± 0.31	0.39 ± 0.03
% of dosed radioactivity undergoing		
primary biodegradation[2)]	96.77 ± 0.12	Not applicable
ultimate biodegradation[3)]	90.87 ± 0.89	Not applicable

SD = Standard deviation
[1)] 100 - (% remaining with solids + % in effluent + % sorbed)
[2)] 100 - (% parent sorbed + % parent in effluent)
[3)] Mineralisation (%) + incorporated into biomass (%)

Table A7_1_2_1_1-6: Distribution of radioactivity (in % of initial dose and µg/L) of the 11.0 µg/L dose level

Triclosan 11.0 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralisation ($^{14}CO_2$)[1)]	75.61 ± 2.41	Not applicable
Remaining with the solids	15.81 ± 2.17	1.74 ± 0.24
Sorbed onto solids as:		
Parent	1.92 ± 0.11	0.21 ± 0.01
Metabolite:		
-0.030	0.14 ± 0.03	0.02 ± 0.00
0.015	1.37 ± 0.17	0.15 ± 0.02
0.653	0.58 ± 0.05	0.06 ± 0.01
Total sorbed	4.01 ± 0.32	0.45 ± 0.03
Present in effluent as:		
Parent	0.64 ± 0.42	0.07 ± 0.05
Metabolite:		
-0.025	1.00 ± 0.18	0.11 ± 0.02
0.031	1.90 ± 0.33	0.21 ± 0.04
0.143	0.70 ± 0.16	0.08 ± 0.02
0.768	0.27 ± 0.07	0.03 ± 0.01
Total effluent	4.58 ± 0.39	0.51 ± 0.06
% of dosed radioactivity undergoing		
primary biodegradation[2)]	97.40 ± 0.39	Not applicable
ultimate biodegradation[3)]	91.41 ± 0.40	Not applicable

SD = Standard deviation
[1)] 100 - (% remaining with solids + % in effluent + % sorbed)
[2)] 100 - (% parent sorbed + % parent in effluent)
[3)] Mineralisation (%) + incorporated into biomass (%)

Table A7_1_2_1_1-7: Distribution of radioactivity (in % of initial dose and µg/L) of the 20.0 µg/L dose level

Triclosan 20.0 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralisation ($^{14}CO_2$)[1)]	76.61 ± 1.75	Not applicable
Remaining with the solids	14.20 ± 0.76	2.84 ± 0.15
Sorbed onto solids as:		
Parent	1.94 ± 0.37	0.39 ± 0.07
Metabolite:		
-0.031	0.09 ± 0.03	0.02 ± 0.01
0.016	0.82 ± 0.12	0.16 ± 0.02
0.694	0.48 ± 0.06	0.10 ± 0.10
Total sorbed	3.34 ± 0.56	0.68 ± 0.11
Present in effluent as:		
Parent	0.90 ± 0.23	0.18 ± 0.05
Metabolite:		
-0.018	1.01 ± 0.33	0.20 ± 0.07
0.033	2.46 ± 0.50	0.49 ± 0.10
0.149	1.22 ± 0.48	0.24 ± 0.10
0.741	0.33 ± 0.07	0.07 ± 0.01
Total effluent	5.86 ± 1.17	1.18 ± 0.23
% of dosed radioactivity undergoing		
primary biodegradation[2)]	97.16 ± 0.61	Not applicable
ultimate biodegradation[3)]	90.80 ± 1.62	Not applicable

SD = Standard deviation
[1)] 100 - (% remaining with solids + % in effluent + % sorbed)
[2)] 100 - (% parent sorbed + % parent in effluent)
[3)] Mineralisation (%) + incorporated into biomass (%)

Table A7_1_2_1_1-8: Distribution of radioactivity (in % of initial dose and µg/L) of the 50.0 µg/L dose level

Triclosan 50.0 µg/L	Mean ± SD (%)	Mean ± SD (µg/L)
Mineralisation ($^{14}CO_2$)[1)]	76.72 ± 2.45	Not applicable
Remaining with the solids	16.08 ± 2.17	8.04 ± 1.09
Sorbed onto solids as:		
Parent	1.38 ± 0.14	0.69 ± 0.07
Metabolite:		
-0.031	0.14 ± 0.04	0.07 ± 0.02
0.012	1.45 ± 0.31	0.73 ± 0.15
0.694	0.22 ± 0.04	0.11 ± 0.02
Total sorbed	3.20 ± 0.51	1.61 ± 0.26
Present in effluent as:		
Parent	0.72 ± 0.21	0.36 ± 0.11
Metabolite:		
-0.023	0.73 ± 0.14	0.37 ± 0.07
0.032	1.84 ± 0.29	0.92 ± 0.15
0.147	0.64 ± 0.21	0.32 ± 0.10
0.682	0.05 ± 0.06	0.03 ± 0.03
Total effluent	4.00 ± 0.60	2.00 ± 0.29
% of dosed radioactivity undergoing		
primary biodegradation[2)]	97.90 ± 0.28	Not applicable
ultimate biodegradation[3)]	92.80 ± 1.11	Not applicable

SD = Standard deviation
[1)] 100 - (% remaining with solids + % in effluent + % sorbed)
[2)] 100 - (% parent sorbed + % parent in effluent)
[3)] Mineralisation (%) + incorporated into biomass (%)

Section A7.1.2.2.1 Annex Point IIIA, 2.2.1	Aerobic aquatic degradation study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	An aerobic aquatic degradation study has not been performed and is not considered as relevant due to the intended use and the waste management of DCPP. The active substance is readily biodegradable and not released directly to freshwater systems. A reliable water/sediment degradation stud y was performed with the structurally similar active substance triclosan (CAS-No. 3380-34-5). The phenol ring of triclosan and DCPP is identical and a similar fate and behaviour of both substances in the environment is to be expected. Since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that the degradation rate of triclosan will be lower compared to DCPP. For this reason, data on triclosan was used to evaluate the distribution and degradation of DCPP in water/sediment systems.	x
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	August 2011	
Evaluation of applicant's justification	*Deletion:* An aerobic aquatic degradation study has not been performed and is not considered as relevant due to the intended use and the waste management of DCPP. The active substance is readily biodegradable and not released directly to freshwater systems.	
Conclusion	Agree with applicant´s version.	
Remarks	-	

Section A7.1.2.2.2 Annex Point IIIA, XII 2.1.	Water/sediment degradation study	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	No study assessing the degradation of DCPP in water/sediment systems was performed. However, a reliable water/sediment degradation study was performed with the structurally similar active substance triclosan (CAS-No. 3380-34-5). The phenol ring of triclosan and DCPP is identical and a similar fate and behaviour of both substances in the environment is to be expected. Since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that the degradation rate of triclosan will be lower compared to DCPP. Thus it is justified to use data on triclosan to evaluate the distribution and degradation of DCPP in water/sediment systems. Please refer to the study summary of triclosan.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	July 2011	
Evaluation of applicant's justification	Agree with applicant's version.	
Conclusion	Agree with applicant's version.	
Remarks	Regarding metabolites similar metabolites are to be expected: For tri-chlorinated metabolites resembling triclosan, di-chlorinated metabolites resembling DCPP are to be expected accordingly.	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA, XII 2.1.

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]D. (2006): ^{14}C-Triclosan: Route and rate of degradation in aerobic aquatic sediment systems.[redacted] Study[redacted]Date: 2006-07-25.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD Guideline for the Testing of Chemicals, Guideline 308, April 2002; EPA Pesticide Assessment Guideline, Subdivision N, Section 162-4, October 1982	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	**Identity: ^{14}C-Triclosan** Chemical name: 5-Chloro-2-(2,4-dichloro-phenoxy)-phenol	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Specific radioactivity: 5.43 MBq/mg	
3.1.3	Purity	Radiochemical purity: 96.0% as determined after purification by RCC Ltd.	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	For each test system, the water and sediment phases were separated and the sediments were extracted with acetonitrile/water (4:1; v/v). Soxhlet extraction was additionally performed on selected samples. The radioactivity in the water phases and sediment extracts was determined by LSC followed by chromatographic analysis of concentrated aliquots with HPLC and 2D-TLC. After room temperature and Soxhlet extractions the duplicate samples of each sediment from day 104 were submitted to harsh extraction procedure. The sediments were extracted with acetonitrile / 0.1 M hydrochloric acid (1:1; v/v) under reflux conditions for about 4 hours. The extracted radioactivity was determined by LSC. The non-extractable residues were submitted to organic matter fractionation.	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA, XII 2.1.

		Basically, this fractionation consists of the extraction of sediment with 0.5 M NaOH solution, and subsequent precipitation of the humic acids by reducing to pH 1. After all extractions, air dried samples were homogenised in a grinder and combusted by an OX 500 sample oxidiser (Zinsser Analytic; Frankfurt/Germany). Radioactivity was quantified by LSC. HPLC was the primary method of chromatographic profiling. The analytical conditions for the analytical methods are described below: Pump: Merck-Hitachi L-6200 or L-6200A Autosampler: Merck-Hitachi AS-2000 or AS-2000A UV-detector: Merck-Hitachi L4000 (224 nm) ^{14}C-detector: Packard Flow scintillation analyser, A500 or 500TR Pre-column: 4 x 4 mm, 5 µm LiChrospher 100 C18 Column: 25 cm x 4.6 mm, 5 µm Kromasil C18 Mobile Phase: Solvent A: 0.1% TFA in water, Solvent B: 0.1% TFA in acetonitrile or Solvent A: 0.1% phosphoric acid in water, Solvent B: acetonitrile Flow: 1.0 ml/min (eluent) ^{14}C-Detection: 1.0 mL of eluent was continuously mixed 2 mL of Flo-Scint A Two dimensional TLC (2D-TLC) analysis was used as a second method of chromatographic profiling and to confirm the HPLC results for selected samples. 2D-TLC was performed on pre-coated plates (20 x 20 cm, layer thickness 0.25 mm) of silica gel 60 F254 (normal phase). The following solvent systems (SS) were used: SS 1: Ethyl acetate/hexane (50:50; v/v) SS 2: Chloroform/methanol/water/acetic acid (75:25:2:2; v/v/v/v)	
3.2	**Reference substance**	Identity: Unlabelled Triclosan Chemical name: [5-Chloro-2-(2,4-dichloro-phenoxy)-phenol] CAS-RN no.: 3380-34-5 Batch no.: 6[redacted] Purity: 99.6%	x
3.2.1	Method of analysis for reference substance	HPLC and 2D-TLC (see point 3.1.5)	
3.3	**Water/sediment systems**	The water/sediment systems were sampled from a river (Rhine river / Mumpf AG Switzerland) and from a pond (Möhlin BL, Switzerland) and consisted of natural water filtered through a 0.2 mm sieve and the uppermost 5-10 cm of the sediment sieved through a 2 mm mesh. The water/sediment characteristics are shown in Tables A7_1_2_2_2-1 and A7_1_2_2_2-2.	
3.4	**Testing procedure**		
3.4.1	Test system	The route and rate of degradation of Triclosan in water/sediment systems was investigated in a laboratory study using radiolabeled ^{14}C-Triclosan. The experiment was performed in open gas-flow-systems in 1000 mL	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA, XII 2.1.

		glass metabolism flasks (inner diameter: approximately 10.6 cm, surface area: approximately 88.2 cm^2). Sediments were added to a depth of approximately 2.0 cm. A water volume of 600 mL was added to reach a depth of approximately 6.5 cm. The sediment / water volume was about 1:3.3. The flasks were incubated under continuous ventilation with moistened air and connected to a series of two volatile traps, the first trap containing 50 mL ethylene glycol and the second trap 50 mL 2 N NaOH. The samples were incubated at 20 ± 2 °C in the dark.
3.4.2	Test solutions and conditions	The test systems were acclimated under aerobic conditions in the dark for about two weeks prior to treatment. During this time, the measured values for pH, oxygen concentration and redox potential in water and pH and redox potential in sediment had reached constant values. Following the acclimation period, the test item was applied to the water phase of the water/sediment systems at a dose of 0.109 mg/L. During the incubation period, a stream of air was allowed to pass through the samples. Organic volatiles and $^{14}CO_2$ were trapped, radioactivity was monitored by LSC at each exchanging interval (two week interval). During the entire incubation period, the samples were incubated in the dark and aerated by gentle agitation of the water layer so that the sediment remained undisturbed. Duplicate samples of each system were taken for analysis after 0, 1, 7, 14, 28, 56 and 104 days of incubation. A total radioactivity balance and the distribution of radioactivity was established for each interval.
		4 RESULTS
4.1	**Water/sediment distribution and metabolism**	In Tables A7_1_2_2_2-3 to A7_1_2_2_2-6, the distribution and metabolism of Triclosan in two water/sediment-systems is shown and expressed in percent of the applied radioactivity.
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The purpose of the study was to determine the degradation rate of ^{14}C-Triclosan in two different aquatic model systems under aerobic conditions.
5.2	**Results and discussion**	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA, XII 2.1.

5.2.1	Distribution	Total recoveries of the applied radioactivity (material balances) averaged 94.1 ± 2.6% and 95.4 ± 1.5% in the river and pond systems, respectively. The radioactivity in the water phase decreased continuously, from mean amounts of 96.9% (river) and 95.2% (pond) immediately after application to 7.9% and 11.4% of the applied radioactivity, respectively, within 14 days of incubation. At the end of incubation (day 104), only 1.7% and 2.7% of the applied radioactivity was detected in the river and pond water phases, respectively. The extractable radioactivity from sediments increased reaching maximum mean amounts of 75.1% after 7 days (river) and 78.7% of the applied radioactivity after 14 days (pond). Corresponding values for day 104 were 34.7% and 27.6%, respectively. Soxhlet extractions recovered up to 6.1% of the applied radioactivity (both systems).
5.2.2	Degradation products	Active Substance In both aquatic systems ^{14}C-Triclosan dissipated very rapidly from the water phase to the sediments. The concentration of the test item in the water phases, represented on average 92.9% (river) and 88.0% (pond) immediately after application decreasing to 3.8% and 6.5% within 14 days of incubation. At the end of incubation, ^{14}C-Triclosan in the water phase reached mean amounts of 0.1% and below detection limit. In the sediment, the test item represented a maximum mean amount of 69.2% (river) and 74.9% (pond) of the applied radioactivity after 7 and 14 days respectively. Thereafter, its concentration declined continuously to about 21% of the applied radioactivity after 104 days. The concentration of ^{14}C-Triclosan in the total river and pond systems represented initially 92.9% and 88.0% and decreased to 21.4% (river) and 21.8% (pond) on day 104. Degradation of the test item was observed in both compartments, but was more pronounced in the aqueous phases than in sediments. Metabolites Besides the test item, up to sixteen minor metabolites were observed, one of which was identified by co-chromatography using HPLC and 2D-TLC as methyl-triclosan (highest amounts of 4.8% on day 104 in river system, sediment extract). All of the other minor metabolites reached highest 6.5% (M8 at day 56 in river) of the applied radioactivity in the water phases or sediments of both systems. At the end of the study (day 104) their amounts were ≤ 5.5%. For further analysis their amounts were by far too low.
5.2.3	CO_2 formation	The mineralization of the test item (dichloro-phenyl ring moiety) reached maximum mean amounts of 21.4% (river) and 29.1% (pond) of the applied radioactivity after 104 days of incubation. Other organic volatile compounds did not exceed 0.6% of the applied radioactivity in both systems.

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA, XII 2.1.

5.2.4	Bound residues	In sediment, the amount of non-extractable radioactivity steadily increased during incubation. At the end of incubation, means of 32.4% and 33.0% of the applied radioactivity remained unextracted from river and pond sediments, respectively. Further harsh extractions using acidic conditions under reflux extracted a maximum of 3.5% of the applied radioactivity from the sediment on day 104. Subsequent organic matter fractionation of the non-extractable residues indicates that in both sediments the major part of the non-extractable radioactivity was bound to the immobile humin fraction amounting to mean amounts of 17.5% and 20.2% of the applied radioactivity for river and pond, respectively. Corresponding values for the fulvic acids were 7.5% and 5.0% and for the humic acids 4.0% and 6.0%.
5.2.5	DT_{50} and DT_{90} values	The dissipation times (DT_{50} and DT_{90}) for the parent compound ^{14}C-Triclosan from the water phase, sediment and total system were summarized in Table A7_1_2_2_2-7. Half-lives were calculated using first order kinetics (ModelMaker® calculation software, vers. 3.04, Cherwell Scientific Publishing Ltd, The Magdalen Centre, Oxford Science Park, Oxford, UK): <u>River</u> Water: DT_{50} = 1.2 days; DT_{90} = 4.0 days Sediment: DT_{50} = 56.4 days; DT_{90} = 187.3 days Total system: DT_{50} = 41.1 days; DT_{90} = 136.5 days <u>Pond</u> Water: DT_{50} = 1.4 days; DT_{90} = 4.5 days Sediment: DT_{50} = 56.3 days; DT_{90} = 186.9 days Total system: DT_{50} = 58.3 days; DT_{90} = 193.7 days

6 CONCLUSION

6.1	**Conclusion**	In aerobic systems, ^{14}C-Triclosan dissipates rapidly from the water phase by degradation and adsorption to the sediment. A mean DT_{50} value of about one day was calculated for both the river and pond water phases. In the sediment, the parent compound was degraded more slowly with DT_{50} values of 56 days for both aquatic systems. Corresponding dissipation half-lives for the total system were 41 (river) and 58 (pond). Degradation of ^{14}C-Triclosan in both compartments proceeded via formation of numerous minor metabolites, one of which was identified as methyl-triclosan, to formation of bound residues and significant radioactive carbon dioxide.
6.1.1	Reliability	1

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	July 2011
Materials and Methods	3.2.: *Addition*: Reference Substance used in Identification: Methyl-Triclosan: [redacted] SEP 2007, >99.6%
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1-2
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Some Measurements of parameters for characterisation of water-sediment samples where not exactly done according to Table provided in OECD test guideline 308: No TOC is reported for the start of the test in the water. In the sediment the pH is not reported at start of acclimation, start of test and during the test. Organic carbon was measured instead of TOC in the sediment. No measurement at posthandling stage and at the end of the test.

Table A7_1_2_2_2-1: Parameters measured for river and pond water phases before sampling at the sampling location, before acclimation and at the end of the incubation period

Water characteristics				
Parameters	Mumpf, AG, Switzerland River		Möhlin, AG, Switzerland Pond	
	Post handling / before acclimation	End of incubation[2]	Post handling / before acclimation	End of incubation[2]
Temperature (°C) Surface 5 cm above sediment	 15.3[1] 15.1[1]	20 ± 2	 14.5[1] 14.0[1]	20 ± 2
pH Surface 5 cm above sediment	 8.1[1] 8.0[1]	8.2	 7.6[1] 7.5[1]	8.4
	7.3[3]		7.2[3]	
Oxygen content (mg/l) Surface 5 cm above sediment	 9.7[1] 9.6[1]	10.2	 5.8[1] 5.4[1]	10.3
	3.2[3]		2.2[3]	
Redox potential	235[1]	104	257[1]	102
	204[3]		210[3]	
TOC (total organic carbon; mg C/l)	2.07[1]	6.77	7.11[1]	9.13
	2.97[3]		6.43[3]	
Hardness (°dH)[1]	12	14	18	15

1: post handling
2: parameters determined in control samples (mean of duplicates) at the end of incubation
3: before acclimation

Table A7_1_2_2_2-2: Sediment parameters determined at the sampling location and at the end of the incubation period

Sediment characteristics				
Parameters	Mumpf, AG, Switzerland River		Möhlin, AG, Switzerland Pond	
	before acclimation	End of incubation[1]	before acclimation	End of incubation[1]
pH ($CaCl_2$)[1]	7.29	7.84	7.26	7.01
Redox potential (mV)[4]	-40	-121	-100	-114
N-total (%)[1]	0.11	0.11	0.41	0.43
P-total (g/kg sediment)[1]	0.37	0.38	0.56	0.53
Organic carbon (g C/100 g dry sediment)	0.78	0.80	5.0	4.91
Microbial biomass (mg microbial C/100 g dry sediment)[4]	60.7[2]	27.9	232.2[2]	129.6
	64.3[3]		130.9[3]	
Cation exchange capacity (mVal/kg dry sediment)[1]	12.1	14.4	31.4	27.8
Dry mass (kg wet sediment/kg dry sediment)[4]	1.55		3.00	
Particle size distribution[1] USDA classification Clay (%; < 2 µm) Silt (%; 2-50 µm) Sand (%; > 50 µm)	 sandy loam 4.1 25.9 70.0		 silty clay loam 31.1 62.4 6.5	

1: Parameters determined by Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA), Speyer, Germany
2: before acclimation
3: prior to application (day 0)
4: Parameters determined at RCC LTD.

Table A7_1_2_2_2-3: Material balance in the river system after treatment with ^{14}C-Triclosan. Values are given in percent of the applied radioactivity

River system Aerobic / 20 °C	Sample	Incubation time in days						
		0	1	7	14	28	56	104
Radioactivity in water	A	97.6	57.2	51.9*	7.8	5.5	4.4	2.4
	B	96.2	56.0	15.1	8.0	5.5	2.0	1.0
	mean	**96.9**	**56.6**	**15.1**	**7.9**	**5.5**	**3.2**	**1.7**
Total radioactivity in sediment	A	< 0.1	41.4	42.5*	82.2	80.7	74.6	69.7
	B	< 0.1	40.5	76.6	85.5	83.4	70.5	64.5
	mean	**< 0.1**	**41.0**	**76.6**	**83.9**	**82.0**	**72.5**	**67.1**
Extractables	A	< 0.1	37.1	39.2*	63.2	50.3	43.0	33.0
	B	< 0.1	37.9	70.8	65.9	59.9	39.5	26.9
Soxhlet	A	n.p.	3.0	1.8*	5.1	5.2	5.8	5.2
	B	n.p.	2.5	4.3	5.3	5.2	6.1	4.4
Total extractables from sediment	mean	< 0.1	40.2	75.1	69.7	60.3	47.2	34.7
Water + Extractables	**mean**	**96.9**	**96.8**	**90.2**	**77.6**	**65.8**	**50.4**	**36.4**
Non-Extractables	A	< 0.1	1.3	1.5	14.0	25.1	25.8	31.5
	B	< 0.1	0.1	1.5	14.3	18.3	24.9	33.2
	mean	**< 0.1**	**0.7**	**1.5**	**14.2**	**21.7**	**25.3**	**32.4**
$^{14}CO_2$	A	n.p.	< 0.1	0.1	3.9	6.9	13.6	19.6
	B	n.p.	< 0.1	0.4	1.6	5.2	18.0	23.2
	mean	**n.p.**	**< 0.1**	**0.4**	**2.7**	**6.1**	**15.8**	**21.4**
Other volatiles in EG	A	n.p.	< 0.1	< 0.1	0.1	0.3	0.3	0.6
	B	n.p.	< 0.1	< 0.1	0.2	0.3	0.3	0.6
	mean	**n.p.**	**< 0.1**	**< 0.1**	**0.1**	**0.3**	**0.3**	**0.6**
Total	**A**	**97.7**	**98.6**	**94.5***	**94.0**	**93.3**	**93.0**	**92.3**
	B	**96.2**	**96.5**	**92.2**	**95.3**	**94.3**	**90.8**	**89.3**
	mean	**97.0**	**97.5**	**92.2**	**94.7**	**93.8**	**91.9**	**90.8**
MEAN +/- SD		**94.1 ± 2.6**						

n.p.: not performed
SD: Standard deviation
*: outlier; not used for mean calculation

Table A7_1_2_2_2-4: Material balance in the pond system after treatment with ^{14}C-Triclosan. Values are given in percent of the applied radioactivity

Pond system Aerobic / 20 °C	Sample	Incubation time in days						
		0	1	7	14	28	56	104
Radioactivity in water	A	94.8	52.9	16.3	11.4	8.7	5.3	2.5
	B	95.7	55.5	18.4	11.5	8.6	5.5	2.8
	mean	**95.2**	**54.2**	**17.4**	**11.4**	**8.7**	**5.4**	**2.7**
Total radioactivity in sediment	A	< 0.1	45.2	79.5	83.7	83.3	75.8	58.5
	B	< 0.1	41.7	78.4	83.6	83.1	77.9	62.9
	mean	**< 0.1**	**43.4**	**79.0**	**83.6**	**83.2**	**76.8**	**60.7**
Extractables	A	< 0.1	40.9	69.7	75.8	64.8	46.4	23.7
	B	< 0.1	34.0	70.2	73.9	62.9	46.3	25.0
Soxhlet	A	n.p.	2.9	5.6	3.7	5.6	4.4	3.2
	B	n.p.	6.1	3.9	4.0	5.7	4.5	3.4
Total extractables from sediment	mean	< 0.1	42.0	74.7	78.7	69.5	50.8	27.6
Water + Extractables	**mean**	**95.2**	**96.2**	**92.1**	**90.1**	**78.2**	**56.2**	**30.3**
Non-Extractables	A	< 0.1	1.4	4.2	4.2	12.9	25.0	31.5
	B	< 0.1	1.5	4.3	5.7	14.5	27.0	34.5
	mean	**< 0.1**	**1.5**	**4.2**	**5.0**	**13.7**	**26.0**	**33.0**
$^{14}CO_2$	A	n.p.	< 0.1	0.2	0.7	2.1	13.3	31.6
	B	n.p.	< 0.1	0.3	0.7	3.6	11.1	26.7
	mean	**n.p.**	**< 0.1**	**0.3**	**0.7**	**2.9**	**12.2**	**29.1**
Other volatiles in EG	A	n.p.	< 0.1	< 0.1	< 0.1	0.2	0.3	0.4
	B	n.p.	< 0.1	< 0.1	< 0.1	0.3	0.2	0.8
	mean	**n.p.**	**< 0.1**	**< 0.1**	**< 0.1**	**0.2**	**0.3**	**0.6**
Total	**A**	**94.8**	**98.1**	**96.1**	**95.8**	**94.3**	**94.7**	**92.9**
	B	**95.7**	**97.2**	**97.2**	**95.8**	**95.6**	**94.7**	**93.2**
	mean	**95.3**	**97.6**	**96.7**	**95.8**	**95.0**	**94.7**	**93.1**
MEAN +/- SD		**95.4 ± 1.5**						

n.p.: not performed
SD: Standard deviation

Table A7_1_2_2_2-5: Distribution of ^{14}C-Triclosan and its metabolites in the water phase, sediment extracts and total river and pond system. Values are given in percent of the applied radioactivity

% of applied radioactivity		Incubation time in days						
		0	1	7	14	28	56	104
Aerobic river (20 °C)								
Triclosan	Water phase (n = 2)	92.9	52.8	11.4	3.8	0.5	0.2	0.1
	sediment extracts (n = 2)	n.a.	39.2	69.2	62.1	51.6	34.2	21.3
	Total system	**92.9**	**92.0**	**80.7**	**66.0**	**52.0**	**34.4**	**21.4**
Methyltriclosan (M7)	Water phase (n = 2)	*	*	*	*	0.1	*	*
	sediment extracts (n = 2)	n.a.	*	0.8	0.8	2.5	3.0	4.8
	Total system	*****	*****	**0.8**	**0.8**	**2.6**	**3.0**	**4.8**
M8	Water phase (n = 2)	*	*	*	*	*	*	*
	sediment extracts (n = 2)	n.a.	*	*	3.1	3.4	6.5	5.5
	Total system	*****	*****	*****	**3.1**	**3.4**	**6.5**	**5.5**
Adsorbed radioactivity**	Water phase (n = 2)	--	--	--	--	--	2.1	0.7
	sediment extracts (n = 2)	n.a.	--	--	--	--	--	--
	Total system	**--**	**--**	**--**	**--**	**--**	**2.1**	**0.7**
Aerobic pond (20 °C)								
Triclosan	Water phase (n = 2)	88.0	48.8	13.5	6.5	2.7	0.1	*
	sediment extracts (n = 2)	n.a.	40.3	71.2	74.9	65.1	44.6	21.8
	Total system	**88.0**	**89.1**	**84.7**	**81.4**	**67.8**	**44.8**	**21.8**
Methyl-triclosan (M7)	Water phase (n = 2)	*	*	*	*	*	*	*
	sediment extracts (n = 2)	n.a.	*	*	*	0.7	2.7	3.4
	Total system	*****	*****	*****	*****	**0.7**	**2.7**	**3.4**
M8	Water phase (n = 2)	*	*	*	*	*	*	*
	sediment extracts (n = 2)	n.a.	*	*	*	*	0.4	0.5
	Total system	*****	*****	*****	*****	*****	**0.4**	**0.5**
Adsorbed radioactivity**	Water phase (n = 2)	--	--	--	--	--	3.3	1.5
	sediment extracts (n = 2)	n.a.	--	--	--	--	--	--
	Total system	**--**	**--**	**--**	**--**	**--**	**3.3**	**1.5**

*: not detected or below detection limit
**: amount of radioactivity bound to particles during the concentration step of the water phase
n.a.: not analysed, total mean radioactivity < 0.1%
--: not applicable

Table A7_1_2_2_2-6: Radioactivity extracted by harsh extraction and distribution of non-extractable radioactivity to the humic and fulvic acids and humin fraction in sediment (river and pond)

Soil organic matter fractionation	Interval day 104, river						Interval day 104, pond					
	Sample A		Sample B		Mean		Sample A		Sample B		Mean	
	%*	%**	%*	%**	%*	%**	%*	%**	%*	%**	%*	%**
Non-extractables[1]	--	31.5	--	33.2	--	32.4	--	31.5	--	34.5	--	33.0
Harsh extractions[2]	--	3.5	--	3.1	--	3.3	--	1.8	--	2.1	--	1.9
Remaining non-extractable	100.0	28.0	100.0	30.1	100.0	29.1	100.0	29.8	100.0	32.5	100.0	31.1
Soluble fraction at low pH (fulvic acids)	26.0	7.3	25.7	7.7	25.8	7.5	15.2	4.5	16.7	5.4	15.9	5.0
Soluble fraction at high pH (humic acids)	13.5	3.8	14.2	4.3	13.9	4.0	19.9	5.9	18.6	6.0	19.2	6.0
Insoluble fraction (humin)	60.5	17.0	60.1	18.1	60.3	17.5	64.9	19.3	64.7	21.0	64.8	20.2
Total	100.0	28.0	100.0	30.1	100.0	29.1	100.0	29.8	100.0	32.5	100.0	31.1
Total (humic acids and humin)	74.0	20.8	74.3	22.4	74.2	21.6	84.8	25.2	83.3	27.1	84.1	26.2

[1]: after cold and Soxhlet extraction
[2]: 0.1 M HCl / acetonitrile (1:1; v/v)
*: values given in % of non-extractable radioactivity
**: values given in % of applied radioactivity

Table A7_1_2_2_2-7: Dissipation of Triclosan in the system water/sediment (river and pond)

Dissipation Time	^{14}C-Triclosan		
	Water Phase	**Sediment**	**Total system**
River			
DT_{50} (days)	1.2	56.4	41.1
DT_{90} (days)	4.0	187.3	136.5
r^2 (correlation coefficient)	0.973	0.973	0.964
Model used	First-order	First-order	First-order
Pond			
DT_{50} (days)	1.4	56.3	58.3
DT_{90} (days)	4.5	186.9	193.7
r^2 (correlation coefficient)	0.974	0.974	0.932
Model used	First-order	First-order	First-order

Section A7.1.3 Adsorption / desorption screening test

Annex Point IIA, VII.7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2007b): Determination of K_{oc} of DCPP according to OECD TG121.[redacted] Report No.[redacted] unpublished, Date: 2007-04-24	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Test Guideline 121	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Non-radiolabelled test substance DCPP	
3.1.1	Lot/Batch number	Lot. No.[redacted] Batch No.:[redacted]	
3.1.2	Specification	Non-radiolabelled test substance	
3.1.3	Purity	Purity by GC: 99.97%; purity by HPLC: 99.94%	
3.1.4	Further relevant properties	-	x
3.1.5	Method of analysis	HPLC-method parameter Column: Phenomenex Luna CN, 250 x 4.6 mm, 5µm, serial no.: 349439 Column oven temp.: 30 °C Solvent: A: methanol B: aqueous citric acid/citrate buffer, pH 6 A:B = 55:45 Flow rate: 1.0 mL/min DA-Detector: 220 nm Injection volume: 2 µL	
3.2	**Degradation products**	Degradation products tested: No (HPLC screening test)	
3.2.1	Method of analysis for degradation products	Not relevant	
3.3	**Reference substance**	Sodium nitrate, 7631-99-4 (assessment of the dead time of the HPLC system) Phenol, 108-95-2 Methyl benzoate, 93-58-3 Naphthalene, 91-20-3	

Section A7.1.3 Adsorption / desorption screening test

Annex Point IIA, VII.7.7

		1,2,3-Trichlorobenzene, 87-61-6 Phenanthrene, 85-01-8 4,4'-DDT, 50-29-3 The purity of the reference substances is given in Table A7_1_3-1.
3.3.1	Method of analysis for reference substance	HPLC analysis (see Point 3.1.5)
3.4	**Soil types**	Not relevant (HPLC method)
3.5	**Testing procedure**	
3.5.1	Test system	The adsorption coefficient K_{oc} of DCPP was assessed by determination of its retention time on a cyanopropyl HPLC column. The cyanopropyl stationary phase is an appropriate medium to simulate soils because it has polar and non-polar parts. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established. Prior determination of the K_{oc}, a calibration graph of reference items, whose K_{oc} has been determined in more precise batch equilibrium experiments, was acquired. Sodium nitrate was used to determine the HPLC system's dead time. The test is based on OECD Test Guideline 121.
3.5.2	Test solution and Test conditions	The preparation of the stock and test solutions of each, the test and the reference items is given in Table A7_1_3-1. About 10 to 20 mg of each item (test substance or reference substance) were filled up with methanol to give stock solutions with a final volume of 1 mL. Out of the stock solutions, 5 to 10 µL were withdrawn and filled up with methanol to a final volume of 1 mL, to give a concentration of 100 µg/mL. 4,4'-DDT was available in a standard stock solution of 5000 µg/mL of methanol. The reference mixture (Ref-Mix) was prepared by combination of the volumes of the reference solutions given in Table A7_1_3-1 and filling up with methanol to a final volume of 1 mL.
3.6	**Test performance**	
3.6.1	Preliminary test	Not performed
3.6.2	Screening test: Adsorption	Initially, each reference substance was injected separately to determine the retention time for identification of the compound in the reference mixture. After the separate injection of the references, the reference mixture (Ref-Mix) was eluted, followed by two injections of the test solution and finally by another injection of the reference mixture. Every solution injected, had a concentration of 100 µg/mL and compound. The amount of every compound introduced into the column was 0.2 µg. For the determination of the capacity factors of the test substance and the reference items, the dead time of the HPLC system was investigated. Therefore, sodium nitrate was eluted, which is not being retarded on the cyanopropyl column, so the retention time of sodium nitrate equals the dead time. The elution was performed in duplicate.
3.6.3	Screening test: Desorption	Not performed
3.6.4	HPLC-method	See Point 3.1.5
3.6.5	Other test	No

Section A7.1.3 Adsorption / desorption screening test

Annex Point IIA, VII.7.7

		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.2	**Screening test: Adsorption**	The retention times and calculated values for k' are summarized in Table A7_1_3-2. The dead time for sodium nitrate (t_0) was determined to amount to 1.8275 min (mean of two measurements). Table A7_1_3-3 summarizes the retention times and corresponding k' values for DCPP. The calculation of the K_{oc} values and the results for DCPP are reported under Point 4.4.	x
4.3	**Screening test: Desorption**	Not performed	
4.4	**Calculations**	$k' = (t_R - t_0)/t_0$ were: k' = capacity factor t_R = retention time in min t_0 = dead time in min $\log (K_{oc}) = \text{slope} \times \log (k') + \text{intercept}$ were: K_{oc} = adsorption coefficient intercept = intercept of linear regression line slope = slope of linear regression line The two DCPP injections gave regression equations of: Injection A: $\log (K_{oc}) = 5.048 \times 0.4502 + 0.8916 = 3.164$ Injection B: $\log (K_{oc}) = 5.048 \times 0.4464 + 0.8916 = 3.145$	
		The final value for the adsorption coefficient log K_{oc} of DCPP is the rounded average of the values determined with injection A and B:	x
		Log K_{oc} (DCPP) = 3.15	x
4.5	**Degradation product(s)**	Not relevant (see Point 3.2)	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A HPLC screening test for assessing the adsorption properties of DCPP was conducted according to OECD Test Guideline121. The test system is described under Point 3.5.1. No relevant deviations from the guideline occurred.	
5.2	**Results and discussion**	The dead time (t_0) with sodium nitrate is 1.8275 min (mean of two measurements).	
		The linear regression of measured k' against K_{oc} values yielded a line with a slope of 5.048, an intercept of 0.8916 and a correlation coefficient of $r^2 = 0.9931$. The capacity factors gained for DCPP amount to 0.4502 and 0.4464.	
		The adsorption coefficient of the test substance was calculated as : log K_{oc} = 3.15 (K_{oc} = 1412.54)	x
5.2.1	Adsorbed a.s. [%]	Not relevant (HPLC screening test)	
5.2.2	K_a	Log k' = 0.4502 (injection A) and 0.4464 (injection B)	
5.2.3	K_d	Not relevant (HPLC screening test)	
5.2.4	Ka_{oc}	log K_{oc} = 3.15 (K_{oc} = 1412.54)	x

Section A7.1.3 Adsorption / desorption screening test

Annex Point IIA, VII.7.7

5.2.5	Ka/Kd	Not relevant (HPLC screening test)	
5.2.6	Degradation products (% of a.s.)	Not relevant (see Point 3.2)	
5.3	**Conclusion**	Based on the results of this screening test and taking into consideration the classification system according to Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973), compounds having K_{oc} values > 690 are considered to be immobile in soils. The test substance DCPP whose K_{oc} value was calculated as 1412.54 can be assumed to be adsorbed in soils and to be less susceptible for translocation.	x
5.3.1	Reliability	1	x
5.3.2	Deficiencies	No	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE (*)

Date	December 2010
Materials and Methods	*3.1.4: Addition: "Dissociation constant*: pKa: 9.49 (according to Study A 3.6/01 "Dissociation constant 2-Hydroxy 4,4'-Dichloro Diphenyl Ether", in Study A 7.1.3 a pKa of 8.2 is reported) ; Water solubility: 19.5 mg/ml at 20°C; Partition coefficient (log Kow): 3.7 at 20°C"
Results and discussion	4.2: *Insertion after second sentence*: The retention time for naphtalene and 1,2,3-trichlorobenzene is identical. 4.4: *Deletion*: Delete term "averaged" 4.4: *Rephrase*: Log Koc (DCPP) = 3.1545
Conclusion	5.2: *Rephrase:* log Koc = 3.1545 (Koc = 1427.25) 5.2.4: *Rephrase:* log Koc = 3.1545 (Koc = 1427.25) *5.3: Rephrase:* Exchange 1412.54 by 1427.25 5.3.1: Change to 2 5.3.2: *Addition:* Poor purity (> 88.9) given for 4,4'-DDT and poor peak width for 4,4'-DDT (> 2seconds) in HPLC chromatogram. The retention time for naphthalene and 1,2,3-trichlorobenzene is identical.
Reliability	2 as this study is a GLP-study conducted according to an OECD Guideline but is flawed by several deficiencies described above.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_1_3-1: Purity of test substance and reference substances as well as stock and test solutions preparation

Substance	Purity (%)	Stock solution (mg/mL)	Volume of stock solution to give 100 µg/mL (µg)*
Sodium nitrate	100.0	16.2	6.17
Phenol	> 98.9	15.8	6.33
Methyl benzoate	100.0	14.4	6.94
Naphthalene	> 98.3	11.1	9.01
1,2,3-Trichlorobenzene	99.9	16.0	6.25
Phenanthrene	> 96.8	14.1	7.09
4,4'-DDT	> 88.9	approx. 5.0	20.00
DCPP	Batch DCS LAB 006 07, Lot 1	10.3	9.71

* Amount of items to give a final concentration of 100 µL/mL in methanol

Table A7_1_3-2:Retention times, capacity factors (k') and Koc values for the reference substances

Substance	t_R Reference mix (min)[1)2)]	k'	Log k'	Log Koc
Phenol	4.0375	1.2093	0.0825	1.32
Methyl benzoate	4.5925	1.5130	0.1798	1.80
Naphthalene	6.5480	2.5830	0.4121	2.75
1,2,3-Trichlorobenzene	6.5480	2.5830	0.4121	3.16
Phenanthrene	9.6035	4.2550	0.6289	4.09
4,4'-DDT	17.7115	8.6917	0.9391	5.63

[1)] t_R = average retention time in min for two measurements of the reference mix
[2)] Dead time for sodium nitrate (t_0) = 1.8275 min (mean of two measurements)

Table A7_1_3-3:Retention times and capacity factors (k') for the test substance DCPP

Injection	t_R DCPP (min)	k'	Log k'
A	6.981	2.8200	0.4502
B	6.936	2.7953	0.4464

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2013). EPI Suite (v4.11, Nov. 2012) calculation for methyl-diclosan (CAS 4640-07-7). [redacted] Date: 2013-05-29 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No	
		Estimation of adsorption coefficient (Koc) by using QSAR method	
2.2	**GLP**	No	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Benzene, 4-chloro-1-(4-chlorophenoxy)-2-methoxy (Methyldiclosan, degradation product of Diclosan)	
3.1.1	Lot/Batch number	Not applicable (estimation method)	
3.1.2	Specification	Not applicable (estimation method)	
3.1.3	Purity	100% (estimation method)	
3.1.4	Further relevant properties	Water solubility: 0.322 mg/L	
3.1.5	Method of analysis	Not applicable (estimation method)	
3.2	**Degradation products**	No	
3.2.1	Method of analysis for degradation products	Not applicable	
3.3	**Reference substance**	Not applicable (estimation method)	
3.3.1	Method of analysis for reference substance	Not applicable (estimation method)	
3.4	**Soil types**	Not applicable (estimation method)	
3.5	**Testing procedure**		
3.5.1	Test system	Not applicable (estimation method)	
3.5.2	Test solution and Test conditions	Not applicable (estimation method)	
3.6	**Test performance**		

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

3.6.1	Screening test: Adsorption	According to (a)"OECD 106": No, estimation method
3.6.2	Screening test: Desorption	Not performed (only estimation of adsorption coefficient, Koc)
3.6.3	HPLC-method	Not applicable (estimation method)
3.6.4	Other test	KOCWIN is part of the estimation program suite EPI Suite v4.11 (US EPA. Estimation Programs Interface Suite for Microsoft Windows, v4.11. United States Environmental Protection Agency, Washington DC, USA. November, 2012). KOCWIN (v2.00) estimates the Koc with two separate estimation methodologies: 1) estimation using first-order Molecular Connectivity Index (MCI) 2) estimation using log Kow (octanol-water partition coefficient) Experimental data for numerous compounds were collected of which 674 were selected for method development after quality control performance. The 674 compounds were eventually divided into a training set of 516 compounds and a validation set of 158 compounds. The training set was divided further into a dataset of 69 non-polar organics and 447 polar organics. For the current model development, the non-polar dataset is designated as compounds having "No Correction Factors" while the polar compounds are designated as compounds "Having Correction factors". From the experimental dataset the following regression equations were derived: 1) MCI method: log Koc = 0.5213 MCI + 0.60 + $\sum P_f N$ 2) Kow method: log Koc = 0.55313 Log Kow + 0.9251 + ΣPfN where $\Sigma P_f N$ is the summation of the products of all applicable correction factor coefficients for polar compounds multiplied by the number of times (N) that factor is counted for the structure.

4 RESULTS

4.1	**Preliminary test**	Not applicable
4.2	**Screening test: Adsorption**	Applicability domain: The substance has a molecular weight of 269.13 g/mol and falls in the range of molecular weights of the training and the validation data set (see Table A7_1_3-3). The maximum number of fragments used for applying a correction factor complies with the number of fragments in the substance. Therefore, the substance is within the applicability domain of the model. According to the QSAR methods the following values were estimated: 1) MCI method: log Koc = 3.51; Koc = 3228 L/kg (see table A7_1 _3-1) 2) Kow method: log Koc = 3.57; Koc = 3718 L/kg (see table A7_1 _3-2)

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA7.7

4.3	**Screening test: Desorption**	Not applicable
4.4	**Calculations**	
4.4.1	Ka , Kd	Not applicable
4.4.2	Ka_{oc} , Kd_{oc}	Not applicable
4.5	**Degradation product(s)**	Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The adsorption coefficient was estimated using two QSAR methods which are included in the KOCWIN program (v2.00) of EPI Suite v4.11. The first method estimates the Koc based on the molecular connectivity index, while the second method is based on the octanol-water partition coefficient. The applicability domain of the model regarding the compound in question has been tested by comparing the molecular weight and the number of fragments of the substance with the statistics of the training and validation data sets of both models.
5.2	**Results and discussion**	The adsorption coefficient (Koc) was estimated to be 3228 L/kg resulting from the MCI method and 3718 L/kg from the Kow method. The results are representative for the uncharged substance.
5.3	**Conclusion**	The substance has a molecular weight of 269.13 g/mol and falls in the range of molecular weights of the training and the validation data set (see Table A7_1_3-3). The maximum number of fragments used for applying a correction factor complies with the number of fragments in the substance. Therefore, the substance is within the applicability domain of the model. The results of both models are very close to each other. Thus the estimated adsorption coefficients can be regarded as reliable for the substance.
5.3.1	Reliability	2 (Scientifically accepted calculation method)
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2013
Materials and Methods	Agree with the Applicant´s version.
Results and discussion	Agree with the Applicant´s version.
Conclusion	Agree with the Applicant´s version.
Reliability	2
Acceptability	Acceptable for environmental risk assessment.
Remarks	-

Table A7_1 _3-1: Details on estimation by MCI method

Parameter	Value
First Order Molecular Connectivity Index	8.186
Non-Corrected Log Koc (0.5213 MCI + 0.60)	4.8671
Fragment Correction(s):	
2 Ether, aromatic (-C-O-C-)	-1.3582
Corrected Log Koc	3.5089
Estimated Koc	**3228 L/kg**

Table A7_1 _3-2: Details on estimation by Kow method

Parameter	Value
Log Kow (KOWWIN v1.68 estimate)	4.58
Non-Corrected Log Koc (0.55313 log Kow + 0.9251)	3.4584
Fragment Correction(s):	
2 Ether, aromatic (-C-O-C-)	0.1118
Corrected Log Koc	3.5703
Estimated Koc	**3718 L/kg**

Table A7_1 _3-3: Applicability domain of MCI and Kow method

Data set	Number of compounds	Range of molecular weights
Training	- With correction: 447 - Without corrections: o MCI: 69 o Kow: 68	32.04 to 665.02 g/mol; average: 224.5 g/mol
Validation	- MCI: 158 - Kow: 150	73.14 to 504.12 g/mol; average: 277.8 g/mol

Section A7.2.1 Annex Point: IIIA XII 1.1	Aerobic degradation in soil	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [X]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	Due to the exclusive indoor application pattern and the proposed use of DCPP in human hygiene products (product type 1), private area and public health area disinfectants and other biocidal products (product type 2) and in food and feed areas (product type 4), potential direct contamination of the environment via the soil pathway is considered negligible. It can be assumed that soil will not be a compartment of concern for DCPP residues.	x, x
	For the environmental exposure assessment (Doc. II-B), default values taken from the TGD for Risk-Assessment (European Commission, 2003) have been used for a very conservative assessment of the soil compartment. The derived soil degradation half-life is considered as very conservative compared with reliable results of two soil laboratory degradation studies performed with structurally similar triclosan (cf. Doc. IIA, 4.1.2.3).	x
	The phenol ring of triclosan and DCPP is identical and a similar fate and behaviour of both substances in the environment is to be expected. Since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that the degradation rate of triclosan in soil will be lower compared to DCPP.	x
	Studies on the aerobic degradation of DCPP in soil have not been performed and are not considered as being necessary in the scope of the EU BPD dossier submission of DCPP.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	September 2011	

Section A7.2.1 Annex Point: IIIA XII 1.1	Aerobic degradation in soil
Evaluation of applicant's justification	*Addition:* Cross at box for "Other existing data" *Deletion:* It can be assumed that soil will not be a compartment of concern for DCPP residues. *Addition:* However, sewage sludge might be applied to soil as fertilizer. Therefore, soil can be contaminated indirectly. Since the compound is eliminated in the sewage treatment plant in a high degree, emissions of DCPP in the sewage sludge are low and thus, only low amounts of the active substance might reach the soil compartment. *Deletion:* For the environmental exposure assessment (Doc. II-B), default values taken from the TGD for Risk-Assessment (European Commission, 2003) have been used for a very conservative assessment of the soil compartment. The derived soil degradation half-life is considered as very conservative compared with reliable results of two soil laboratory degradation studies performed with structurally similar triclosan (cf. Doc. IIA, 4.1.2.3). *Addition:* However, two aerobic degradation studies have been performed with the structurally similar active substance triclosan (CAS-No. 3380-34-5).
Conclusion	Studies on the aerobic degradation of DCPP in soil have not been performed and are not considered as being necessary in the scope of the EU BPD dossier submission of DCPP. However, two aerobic degradation studies have been performed and submitted with the structurally similar active substance triclosan (CAS-No. 3380-34-5). A similar fate and behaviour of both substances in the environment is to be expected. Since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that the degradation rate of triclosan in soil will be lower compared to DCPP. Regarding metabolites similar metabolites are to be expected: For tri-chlorinated metabolites resembling triclosan, di-chlorinated metabolites resembling DCPP are to be expected accordingly. Thus, it is considered justified to bridge the results of the study performed with triclosan to DCPP.
Remarks	-

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2007): ^{14}C-Triclosan: Degradation and metabolism in three soils incubated under aerobic conditions. [redacted] [redacted] Study Number [redacted] Date: 2007-07-XX	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes. Commission Directive 95/36/EC (1995), amending Council Directive 91/414/EEC, Annex I, 7.1.1.1 route of degradation, 7.1.1.2 rate of degradation, 7.1.1.2.1 laboratory studies – aerobic degradation. OECD Guideline 307 (2002): Aerobic and Anaerobic Transformation in Soil.	
2.2	**GLP**	Yes	
2.3	**Deviations**	Deviations are not expected to alter the results of the study	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabelled test substance: ^{14}C-labelled Triclosan [5-Chloro-2-(2,4-dichlorophenoxy)-phenol]	
3.1.1	Lot/Batch number	Lot No.: [redacted]	
3.1.2	Specification	Specific radioactivity: 5.43 MBq/mg	
3.1.3	Purity	Radiochemical purity: 87.3%. The purity was purified before use and re-analysed by HPLC. Its purity was re-determined to be 95.8%	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	<u>HPLC analysis:</u> HPLC was the primary method for quantification of Triclosan and its degradation products. Instruments: Pump: Merck-Hitachi L-6200 Autosampler: Merck-Hitachi AS-2000 UV-detector: Merck-Hitachi L-4000 (224 nm) ^{14}C-detector: Packard Flow scintillation analyser, 500TR Operating conditions: Pre-column: 4 x 3 mm, 5 µm Phenomenex C18 Column: 250 x 4.6 mm, 5 µm Kromasil 100-C18 Column Temperature: 40 °C Solvent A: 0.1% phosphoric acid in water	

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

Solvent B: Acetonitrile
Flow: 1 mL/min

Gradient:	Time (min.)	0	20	40	50	55	55.1	70
	Solvent A (%)	90	35	35	10	10	90	90
	Solvent B (%)	10	65	65	90	90	10	10

Injected Volume: up to 350 µL
^{14}C-detection: 1 mL of eluent was continuously mixed with 2 mL of Flo-Scint A (Packard Instrument)

TLC:
Used as secondary method to confirm the results obtained by HPLC. Two-dimensional TLC (2D-TLC) was performed on pre-coated plates (20 x 20 cm; layer thickness 0.25 mm) of silica gel 60 F_{254} (normal phase). Samples were mixed with the unlabelled reference items and the mixture was applied to the plate by using a Linomat. The mixed reference items were also spotted near the radioactive band to be able to distinguish them from the matrix. The plates were developed with chamber saturation.

The following solvent systems (SS) were used in the study:

SS 1:	Ethyl acetate/hexane	(50/50; v/v)	1st dimension
SS 2:	Chloroform/methanol/ water/acetic acid	(75/25/2/2; v/v/v/v)	2nd dimension
SS 3:	Chloroform/methanol/ water/acetic acid	(70/30/2/2; v/v/v/v)	2nd dimension
SS 4:	Ethyl acetate/hexane	(40/60; v/v)	2nd dimension

Detection:
Non-labelled reference items:
Visualisation by quenching of UV-light at a wavelength of 254 nm

Radioactive zones on TLC plates:
All TLC plates were submitted to phosphor imaging, which was performed on a Fuji BAS 1500. The phosphor imager detects radioactive areas on TLC plates by exposure against a stored phosphor imaging plate. The imaging plate has an image-sensing layer made up of photostimulable phosphor crystals ($BaFBr:Eu^+$). When exposed, this sensing layer accumulates and stores the irradiated radioactive energy. Following exposure, the imaging plate was inserted into an image reading unit for scanning with a laser beam which causes the emission of luminescence in proportion to the adsorbed radiation intensity. The luminescence is detected by a photomultiplier tube and converted to electrical signals.

3.2	**Reference substance**	Unlabelled Triclosan, Batch No.: [redacted], Purity: 99.3% Methyl-Triclosan, Lot No.:[redacted] Purity: 99.6%
3.2.1	Method of analysis for reference substance	Please refer to 3.1.5.
3.3	**Soil types**	Three soil types were used, see table A7.2.1_1
3.4	**Testing procedure**	

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

3.4.1	Test system	Samples of 100 g soil based on the dry weight were incubated under aerobic conditions in all-glass metabolism flasks (inner diameter: about 10.6 cm, volume: ca. 1 litre) in the dark at 20 ± 2 °C or 10 ± 2 °C. The flasks were equipped with an air inlet and outlet. Each system was continuously ventilated with moistened air. For each flask, the exiting air was passed through a trapping system, equipped with two absorption traps containing 50 mL of 2N sodium hydroxide and 50 mL of ethylene glycol to trap $^{14}CO_2$ and organic volatiles, respectively. For sampling on day 0, no absorption traps were set up. The soil moisture content was held at pF between 2.0 and 2.5 throughout the study. Soil water losses were kept to a minimum by circulating moistened air at a minimum volumetric flow rate. To compensate for the water lost by evaporation, an amount of water equal to that lost by evaporation was added, followed by mixing.	
3.4.2	Test solution and Test conditions	<u>Preparation of stock and application solutions:</u> Radiolabelled stock solution: ^{14}C-Triclosan was purified by column chromatography before use. The purified fractions were concentrated under a steam of nitrogen and re-dissolved in 5 mL acetone (stock solution). Non-radiolabelled stock solution: For the high dose application, a solution containing non-radiolabelled Triclosan was prepared. For this purpose, 31.72 mg Triclosan were weighed into a volumetric flask and filled to a final volume of 50 mL with acetone. The concentration of this solution was calculated to be 0.63 mg a.s./mL, corrected for its purity. Normal dose application solution: The application solution for the normal dose samples was prepared by diluting an aliquot of 2.3 mL of the radiolabelled stock solution with acetone to reach a final volume of 30 mL. Its concentration was determined as follows: triplicate aliquots of 300 µL application solution were separately diluted to 10 mL with acetonitrile then triplicate sub-samples of 100 µL of each dilution were measured by LSC. Based on the mean radioactivity measured (63’143 dpm per 100 µL dilution) and the known specific radioactivity, the amount of test item in the application solution was calculated to be 1.94 mg Triclosan/30 mL. High dose application solution: The application solution for the high dose samples was prepared by diluting an aliquot of 370 µL of the radiolabelled stock solution (or 0.33 mg) with 4.63 mL of the non-radiolabelled stock solution (or 2.93 mg) to reach a total volume of 5 mL. The concentration of the high dose application solution was calculated to 3.25 mg Triclosan/5 mL. In order to reach the target concentration of 0.20 mg/100 g dry soil, a volume of 300 µL of the corresponding application solution was calculated to be applied per high dose sample. <u>Treatment and sampling:</u> The freshly sampled soils were sieved (2 mm) prior to use. Aliquots of 100 g dry soil were treated with the ^{14}C-labelled test item at the maximum expected environmental concentration of about 0.2 mg/kg dry	x

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

soil. The soil samples were incubated at 20 ± 2 °C or 10 ± 2 °C in the dark under continuous ventilation with moistened air. The exiting air was passed through a trapping system consisting of flasks of sodium hydroxide and ethylene glycol in series. Prior to treatment and at the end of the incubation period, the microbial biomass was determined for each soil. The results showed that the soils were viable during the study.

Duplicate samples per soil incubated at 20 °C were taken for extraction and analysis immediately after treatment (time 0), and after 1, 2, 3, 7, 14, 28, 61 and 124 days of incubation. For the 10 °C part (soil I), duplicate samples were taken on days 0, 3, 7, 14, 28, 61 and 124 only. All soil samples were exhaustively extracted with acetonitrile/water (4:1; v/v). From the second interval onwards, additional Soxhlet extraction using the same solvent mixture for four hours was performed. The extracts were then concentrated under reduced pressure and analysed by HPLC and/or 2D-TLC for the test item and degradation products. In order to investigate the non-extractable residues, the samples from day 124 (20 °C) were submitted to additional reflux extraction using acidic conditions followed by organic matter fractionation.

A total balance of radioactivity, the nature of extracted radioactivity and pattern of metabolites were established for each sampling interval.

4 RESULTS

4.1 Aerobic soil metabolism

The total mean recovery in terms of percent of the applied radioactivity was 97.6 ± 3.0% (soil I, 20 °C), 97.5 ± 3.1% (soil II), 97.5 ± 2.5% (soil III) and 97.3 ± 2.5% (soil I, 10 °C).

The amount of total extractable radioactivity decreased rapidly during the first 3 days of incubation from between 95% and 99% of the applied radioactivity immediately after treatment (time 0) to 38%, 50% and 42% for soils I (20 °C), II and III, respectively, on day 3. At end of the study (day 124), extractables represented between 13% and 17% of applied for the corresponding soils. For soil I incubated at the lower incubation temperature, the amount of extractable radioactivity was slightly higher, accounting for 74% and 31% on days 3 and 124, respectively. Soxhlet extraction released an individual amount of up to 6% of applied.

See Tables A7.2.1_2 to A7.2.1_10.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The route and rate of degradation of ^{14}C-Triclosan, i.e. [5-Chloro-2-(2,4-dichlorophenoxy)-phenol], were investigated in three soils incubated under aerobic conditions at 20 °C for a period of 124 days. The following soils were used: soil I (Speyer 5M, sandy loam), soil II (Senozan, clay loam) and soil III (Gartenacker, loam). In order to investigate the influence of temperature on the degradation of Triclosan, one of the selected soils (soil I, Speyer 5M) was additionally incubated at 10 °C.

5.2 Results and discussion

5.2.1 DT50 values

The following DT_{50}, DT_{75} and DT_{90} values were calculated for ^{14}C-Triclosan and its major metabolite, Methyl-Triclosan, based on first-

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

order two-compartment (Triclosan) or consecutive first-order kinetics (Methyl-Triclosan):

	Triclosan			Methyl-Triclosan		
	DT_{50}	DT_{75}	DT_{90}	DT_{50}	DT_{75}	DT_{90} [d]
Soil I (20 °C)	2.46	6.16	19.1	96.7	193	321
Soil II (20 °C)	3.28	7.45	25.7	153	307	509
Soil III (20 °C)	2.68	9.26	21.6	39.2	78.3	130
Soil I (10 °C)	10.7	57.1	231	n.c.	n.c.	n.c.

(n.c. = not calculated due to an insufficient number of data points)

5.2.2 Degradation products (% of a.s.)

The amount of ^{14}C-Triclosan and degradates present in the soil extracts was based on chromatographic profiling by HPLC. TLC analysis was used on selected samples in order to confirm the HPLC results.

The amount of Triclosan rapidly decreased with time in all three soils. Immediately after treatment (time 0), the parent compound represented between 92% and 95% of the applied radioactivity. On day 3, it had declined to levels of 36%, 47% and 42% in soils I (20 °C), II and III, respectively. At the end of the study (day 124), it was either not detected (soil II) or present in very low amounts (1% to 4% of applied in soils I and III).

At the lower incubation temperature, the parent compound represented 73% and 17% of the applied radioactivity on days 3 and 124, respectively.

Besides the parent compound, up to three radioactive fractions were formed including the major characterised one, Methyl-Triclosan. Methyl-Triclosan (M3) was present from day 2 onwards and reached peak levels of 18% (day 28), 24% (day 28) and 13% (day 14) in soils I (20 °C), II and III, respectively. In soil I incubated at 10 °C, its rate of formation was slower, with its peak of 15% reached on day 61. By the end of the study, its rate of formation had decreased in all three soils at 20 °C by 7 to 9% of applied. In soil I, the rate slightly declined (by 1% of applied) until day 124. x

All other radioactive fractions were transient, not exceeding 5% of the applied radioactivity at any sampling interval.

See Figure 1 for a pathway of degradation pathway of Triclosan in soil.

5.2.3 Bound residues

The amount of non-extractable radioactivity was very high in all three soils. Bound residues reached peak values of 70%, 61% and 76% of the applied radioactivity in soils I (20 °C), II and III, respectively, throughout the 124-day incubation period. For soil I at 10 °C, an equally high amount of bound residues were formed (max. 60% of applied). Only a small fraction of the bound residues was extracted using acidic harsh extraction under reflux (max. 2-3% of applied). Subsequent organic matter fractionation of the non-extractable residues indicated the major part of the non-extractable radioactivity was bound to the immobile humic acids and humins amounting to 49% to 58% of the applied radioactivity for all three soils. The corresponding range for fulvic acids was 11-15% of applied.

5.2.4 CO_2 formation

Some mineralisation of the radioactive residues was observed in all soils, with radiolabelled carbon dioxide reaching maximum levels of 14%, 16%, 12% and 5% in soils I (20 °C), II, III and I (10 °C), respectively, on

Section A7.2.1 Aerobic degradation in soil (01)

Annex Point: IIIA XII 1.1

		day 124. Volatile products other than $^{14}CO_2$ did not exceed 0.1% of the applied radioactivity for all soils used.
5.3	**Conclusion**	The route and rate of degradation of ^{14}C-Triclosan were investigated in three soils incubated at 20 °C and one soil at a lower incubation temperature of 10 °C. The test item rapidly degraded rapidly in soil with half-lives ranging from 2.5 to 3.3 days at 20 °C. At the lower incubation temperature, a half-life of 11 days was calculated. One major metabolite was formed, Methyl-Triclosan, accounting for up to 24% of the applied radioactivity at day 28 and decreased until study termination (day 124). Half-lives of Methyl-Triclosan at 20 °C were calculated to range from 39 to 153 days depending on the soil type. Aerobic degradation of ^{14}C-Triclosan in soil proceeds primarily via the formation of Methyl-Triclosan and significant amounts of bound residues. Mineralisation of the radioactive residues could be observed.
5.3.1	Reliability	1
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2011
Materials and Methods	3.4.2: Exchange =.20 by 0.02.
Results and discussion	Agree with applicant´s version.
Conclusion	5.2.2: Exchange 7 by 6.
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	The soils were not amended with sewage sludge as foreseen by OECD Guideline 307 when the major route of entry in soil is through sewage sludge).

Table A7.2.1_1: Classification and physico-chemical properties of the soils used

Parameters[1)]			Soils		
			I Speyer 5M	II Senozan	III Gartenacker
Site location:			Germany	France	Germany
Batch:			F5M4806	12/06	12/06
Soil characteristics:					
- pH ($CaCl_2$)			7.10	6.85	7.30
- Organic carbon	(g/100 g soil)	%	1.39	1.04	1.73
- Organic matter*	(g/100 g soil)	%	2.4	1.79	2.98
- Cation exchange capacity	(meq/100 g soil)		13.0	19.5	12.6
- Nitrogen content	(g/100 g soil)	%	n.a.	0.12	0.15
- $CaCO_3$	(g/100 g soil)	%	n.a.	0.70	6.8
Soil type (according to USDA [3]):			Silty sand	Clay loam	Loam
Particle size analyses (mm) USDA:					
< 0.002	(clay)	%	10.7	28.4	10.1
0.002-0.05	(silt)	%	28.9	51.2	47.6
> 0.05	(sand)	%	60.4	20.4	42.3
Max. water holding capacity (MWC)					
(g water/100 g soil)					
at pF 1.0			41.6	52.3	62.8
at pF 2.0			n.a.	30.6	39.5
at pF 2.5			n.a.	22.8	25.0
Biomass** (mg microbial C/100 g dry soil)					
Start of incubation			16.9	19.7	17.7
End of incubation			10.6	10.0	10.8

[1] Parameters determined by Agrolab, Ebikon, Switzerland (non-GLP) or LUFA, Speyer, Germany (GLP).
* Calculated based on the equation: %OM = %OC x 1.724
** Determined by RCC Ltd within the present study
n.a.: Not available, to be added

Table A7.2.1_2: Balance of the applied radioactivity in soil I (Speyer 5M) at 20 °C. Values in percent of applied radioactivity

^{14}C-Triclosan Soil I (sandy loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Extractables[1]	A	99.4	68.0	49.1	30.7	23.8	24.3	21.0	16.1	12.8
	B	98.2	68.8	60.5	35.3	30.7	14.9	19.6	17.2	12.0
	mean	**98.8**	**68.4**	**54.8**	**33.0**	**27.2**	**19.6**	**20.3**	**16.6**	**12.4**
Soxhlet[2]	A	n.p.	3.7	5.3	5.0	4.0	3.2	3.6	3.7	3.6
	B	n.p.	2.9	3.6	5.0	4.9	3.8	2.3	4.4	4.0
	mean	**n.p.**	**3.3**	**4.5**	**5.0**	**4.4**	**3.5**	**3.0**	**4.1**	**3.8**
Total Extractables	A	99.4	71.7	54.4	35.6	27.8	27.5	24.6	19.7	16.4
	B	98.2	71.7	64.2	40.3	35.6	18.7	21.9	21.6	16.0
	mean	**98.8**	**71.7**	**59.3**	**38.0**	**31.7**	**23.1**	**23.3**	**20.7**	**16.2**
Non-Extractables	A	1.9	27.9	44.2	54.4	66.4	65.9	63.1	64.9	65.7
	B	1.8	28.5	34.3	51.4	58.1	74.6	65.2	65.6	66.8
	mean	**1.9**	**28.2**	**39.2**	**52.9**	**62.3**	**70.3**	**64.1**	**65.2**	**66.3**
$^{14}CO_2$	A	n.p.	0.3	1.2	0.3	3.6	6.0	8.2	10.7	15.4
	B	n.p.	0.6	0.9	0.1	3.7	5.7	8.5	11.4	12.7
	mean	**n.p.**	**0.5**	**1.0**	**0.2**	**3.6**	**5.9**	**8.3**	**11.0**	**14.0**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
T O T A L	**A**	**101.3**	**99.8**	**99.8**	**90.4**	**97.8**	**99.5**	**95.9**	**95.3**	**97.6**
	B	**100.1**	**100.8**	**99.4**	**91.9**	**97.4**	**99.1**	**95.7**	**98.7**	**95.7**
MEAN +/- SD					**97.6**	±	**3.0**			

1 Extracted with acetonitrile / water (4/1; v/v) up to four times
2 Soxhlet extraction with acetonitrile / water (4/1; v/v) for four hours
n.p. Not performed
SD Standard deviation

Table A7.2.1_3: Balance of the applied radioactivity in soil II (Senozan). Values in percent of applied radioactivity

^{14}C-Triclosan Soil II (clay loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Extractables[1]	A	98.1	75.3	59.4	46.6	38.2	27.1	24.2	18.9	15.4
	B	97.1	75.1	62.8	45.6	36.3	30.2	26.2	22.1	14.9
	mean	**97.6**	**75.2**	**61.1**	**46.1**	**37.2**	**28.7**	**25.2**	**20.5**	**15.2**
Soxhlet[2]	A	n.p.	2.7	3.0	4.4	4.2	3.3	4.3	2.6	2.4
	B	n.p.	2.3	2.5	4.2	6.3	3.2	4.1	2.6	1.9
	mean	**n.p.**	**2.5**	**2.7**	**4.3**	**5.2**	**3.3**	**4.2**	**2.6**	**2.2**
Total Extractables	A	98.1	78.1	62.4	50.9	42.4	30.5	28.6	21.5	17.8
	B	97.1	77.4	65.2	49.7	42.6	33.4	30.3	24.7	16.8
	mean	**97.6**	**77.7**	**63.8**	**50.3**	**42.5**	**31.9**	**29.4**	**23.1**	**17.3**
Non-Extractables	A	2.0	22.0	37.3	43.6	48.3	61.1	60.0	59.5	60.7
	B	2.1	23.0	34.2	44.5	53.5	58.3	59.3	56.8	60.9
	mean	**2.1**	**22.5**	**35.7**	**44.0**	**50.9**	**59.7**	**59.6**	**58.1**	**60.8**
$^{14}CO_2$	A	n.p.	<0.1	0.9	<0.1	4.4	6.8	9.8	11.6	18.5
	B	n.p.	<0.1	1.3	<0.1	4.1	7.4	9.2	11.6	14.0
	mean	**n.p.**	**<0.1**	**1.1**	**<0.1**	**4.2**	**7.1**	**9.5**	**11.6**	**16.2**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.1**
T O T A L	**A**	**100.1**	**100.2**	**100.6**	**94.6**	**95.1**	**98.4**	**98.4**	**92.7**	**97.1**
	B	**99.2**	**100.5**	**100.8**	**94.3**	**100.2**	**99.2**	**98.8**	**93.1**	**92.0**
MEAN +/- SD					**97.5**	±	**3.1**			

1 Extracted with acetonitrile / water (4/1; v/v) up to four times
2 Soxhlet extraction with acetonitrile / water (4/1; v/v) for four hours
n.p. Not performed
SD Standard deviation

Table A7.2.1_4: Balance of the applied radioactivity in soil III (Gartenacker). Values in percent of applied radioactivity

^{14}C-Triclosan Soil III (loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Extractables[1]	A	96.6	65.9	48.9	39.2	39.2	21.0	17.4	10.1	9.4
	B	94.1	65.3	48.4	37.9	26.1	21.5	13.2	9.0	10.5
	mean	**95.3**	**65.6**	**48.7**	**38.6**	**32.7**	**21.2**	**15.3**	**9.6**	**10.0**
Soxhlet[2]	A	n.p.	4.4	3.8	4.1	4.1	4.6	4.3	2.5	2.3
	B	n.p.	4.4	4.5	3.3	4.1	4.8	5.0	1.7	2.8
	mean	**n.p.**	**4.4**	**4.1**	**3.7**	**4.1**	**4.7**	**4.7**	**2.1**	**2.5**
Total Extractables	A	96.6	70.3	52.7	43.4	43.4	25.6	21.7	12.7	11.7
	B	94.1	69.7	52.9	41.2	30.2	26.3	18.2	10.7	13.3
	mean	**95.3**	**70.0**	**52.8**	**42.3**	**36.8**	**25.9**	**20.0**	**11.7**	**12.5**
Non-Extractables	A	4.3	29.4	44.8	50.2	50.2	69.0	69.1	73.6	78.4
	B	4.2	29.8	45.5	52.1	66.2	67.9	73.6	74.8	73.2
	mean	**4.2**	**29.6**	**45.1**	**51.1**	**58.2**	**68.5**	**71.3**	**74.2**	**75.8**
$^{14}CO_2$	A	n.p.	<0.1	1.1	1.2	2.8	<0.1	6.2	5.4	9.8
	B	n.p.	<0.1	1.2	1.3	3.0	<0.1	5.9	9.8	13.2
	mean	**n.p.**	**<0.1**	**1.1**	**1.2**	**2.9**	**<0.1**	**6.0**	**7.6**	**11.5**
Organic Volatiles	A	n.p.	<0.1	<0.1	1.1*	<0.1	<0.1	<0.1	<0.1	0.7*
	B	n.p.	<0.1	<0.1	1.2*	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**1.1***	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.4***
T O T A L	**A**	**100.9**	**99.9**	**98.6**	**95.8**	**96.5**	**94.7**	**97.0**	**91.7**	**100.5**
	B	**98.2**	**99.6**	**99.5**	**95.8**	**99.4**	**94.2**	**97.8**	**95.4**	**99.9**
MEAN +/- SD					**97.5**	±	**2.5**			

1 Extracted with acetonitrile / water (4/1; v/v) up to four times
2 Soxhlet extraction with acetonitrile / water (4/1; v/v) for four hours
n.p. Not performed
SD Standard deviation
* This was probably only due to a contamination of the trapping flask since no continuous increase was observed.

Table A7.2.1_5: Balance of the applied radioactivity in soil I (Speyer 5M) at 10 °C. Values in percent of applied radioactivity

^{14}C-Triclosan Soil I at 10°C (% applied)	Duplicate	INCUBATION TIME IN DAYS						
		0	3	7	14	28	61	124
Extractables[1]	A	97.0	71.4	52.4	39.5	34.8	26.6	24.0
	B	98.7	70.2	59.2	38.7	40.0	35.4	29.7
	mean	**97.9**	**70.8**	**55.8**	**39.1**	**37.4**	**31.0**	**26.9**
Soxhlet[2]	A	n.p.	2.9	3.8	4.8	3.0	3.8	3.2
	B	n.p.	3.7	4.9	4.4	2.5	3.9	4.6
	mean	**n.p.**	**3.3**	**4.4**	**4.6**	**2.8**	**3.8**	**3.9**
Total Extractables	A	97.0	74.3	56.3	44.3	37.8	30.4	27.2
	B	98.7	73.9	64.2	43.1	42.5	39.3	34.3
	mean	**97.9**	**74.1**	**60.2**	**43.7**	**40.1**	**34.8**	**30.8**
Non-Extractables	A	1.9	23.2	40.9	53.9	54.9	61.3	63.7
	B	1.9	17.6	34.8	52.9	51.9	52.8	55.6
	mean	**1.9**	**20.4**	**37.8**	**53.4**	**53.4**	**57.1**	**59.6**
$^{14}CO_2$	A	n.p.	<0.1	1.2	2.4	3.2	3.6	4.9
	B	n.p.	0.1	1.1	2.4	2.6	3.8	5.3
	mean	**n.p.**	**<0.1**	**1.2**	**2.4**	**2.9**	**3.7**	**5.1**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
T O T A L	**A**	**98.9**	**97.6**	**98.4**	**100.6**	**95.9**	**95.4**	**95.8**
	B	**100.6**	**91.7**	**100.0**	**98.5**	**97.1**	**95.8**	**95.3**
MEAN +/- SD				**97.3**	±	**2.5**		

1 Extracted with acetonitrile / water (4/1; v/v) up to four times
2 Soxhlet extraction with acetonitrile / water (4/1; v/v) for four hours
n.p. Not performed
SD Standard deviation

Table A7.2.1_6: Distribution of non-extractable radioactivity to the humic and fulvic acids and humin fraction in soils I (top), II (middle) and III (bottom)

Harsh extraction and soil organic matter fractionation	Interval day 124, Soil I at 20°C					
	Replicate A		Replicate B		Mean	
	%*	%**	%*	%**	%*	%**
Non-extractables (after cold and Soxhlet extraction)	--	65.7	--	66.8	--	66.3
Harsh extractions (0.1 M HCl / acetonitrile (1:1; v/v))	--	2.7	--	2.3	--	2.5
Remaining non-extractable	**100.0**	**63.1**	**100.0**	**64.5**	**100.0**	**63.8**
Soluble fraction at low pH (fulvic acids)	18.7	11.8	15.9	10.2	17.3	11.0
Soluble fraction at high pH (humic acids)	31.6	19.9	26.4	9.5	29.0	14.7
Insoluble fraction (humin)	49.7	31.4	57.8	37.2	53.7	34.3
Total	100.0	63.1	100.0	64.5	100.0	63.8
Total (humic acids and humin)	81.3	51.3	84.1	46.8	82.7	49.0

Harsh extraction and soil organic matter fractionation	Interval day 124, Soil II at 20°C					
	Replicate A		Replicate B		Mean	
	%*	%**	%*	%**	%*	%**
Non-extractables (after cold and Soxhlet extraction)	--	60.7	--	60.9	--	60.8
Harsh extractions (0.1 M HCl / acetonitrile (1:1; v/v))	--	2.5	--	2.2	--	2.4
Remaining non-extractable	**100.0**	**58.2**	**100.0**	**58.7**	**100.0**	**58.5**
Soluble fraction at low pH (fulvic acids)	19.9	11.6	19.6	11.5	19.8	11.6
Soluble fraction at high pH (humic acids)	17.0	9.9	16.2	20.6	16.6	15.3
Insoluble fraction (humin)	63.1	36.7	64.2	37.7	63.6	37.2
Total	100.0	58.2	100.0	58.7	100.0	58.5
Total (humic acids and humin)	80.1	46.6	80.4	58.3	80.2	52.5

Harsh extraction and soil organic matter fractionation	Interval day 124, Soil III at 20°C					
	Replicate A		Replicate B		Mean	
	%*	%**	%*	%**	%*	%**
Non-extractables (after cold and Soxhlet extraction)	--	78.4	--	60.9	--	69.6
Harsh extractions (0.1 M HCl / acetonitrile (1:1; v/v))	--	2.7	--	2.2	--	2.5
Remaining non-extractable	**100.0**	**75.6**	**100.0**	**58.7**	**100.0**	**67.2**
Soluble fraction at low pH (fulvic acids)	20.4	15.4	21.3	15.1	20.8	15.2
Soluble fraction at high pH (humic acids)	30.1	22.8	29.1	20.6	29.6	21.7
Insoluble fraction (humin)	49.5	37.4	49.6	35.1	49.5	36.3
Total	100.0	75.6	100.0	70.8	100.0	73.2
Total (humic acids and humin)	79.6	60.2	78.7	55.7	79.2	58.0

*: Normalised values
**: Values expressed in % of applied radioactivity
HCl: Hydrochloric acid

Table A7.2.1_7: Pattern of degradation and formation of metabolites in soil I (Speyer 5M) at 20°C. Values in percent of applied radioactivity

^{14}C-Triclosan Soil I (sandy loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Parent	A	95.2	70.7	52.7	32.8	19.9	12.1	6.1	4.2	3.9
	B	94.2	70.5	63.0	39.0	26.2	12.5	5.4	4.2	4.6
	mean	**94.7**	**70.6**	**57.9**	**35.9**	**23.1**	**12.3**	**5.8**	**4.2**	**4.2**
M1 (unknown)	A	4.1	1.0	0.4	*	*	*	*	*	*
	B	4.0	1.1	1.2	*	*	*	*	*	*
	mean	**4.1**	**1.1**	**0.8**	*****	*****	*****	*****	*****	*
M3 (methyl-triclosan)	A	*	*	1.3	2.9	7.9	15.3	18.5	15.5	12.5
	B	*	*	*	1.3	9.3	6.2	16.5	17.5	11.4
	mean	*****	*****	**0.7**	**2.1**	**8.6**	**10.8**	**17.5**	**16.5**	**12.0**
Non-Extractables	A	1.9	27.9	44.2	54.4	66.4	65.9	63.1	64.9	65.7
	B	1.8	28.5	34.3	51.4	58.1	74.6	65.2	65.6	66.8
	mean	**1.9**	**28.2**	**39.2**	**52.9**	**62.3**	**70.3**	**64.1**	**65.2**	**66.3**
$^{14}CO_2$	A	n.p.	0.3	1.2	0.3	3.6	6.0	8.2	10.7	15.4
	B	n.p.	0.6	0.9	0.1	3.7	5.7	8.5	11.4	12.7
	mean	**n.p.**	**0.5**	**1.0**	**0.2**	**3.6**	**5.9**	**8.3**	**11.0**	**14.0**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
TOTAL	**A**	**101.3**	**99.8**	**99.8**	**90.4**	**97.8**	**99.5**	**95.9**	**95.3**	**97.6**
	B	**100.1**	**100.8**	**99.4**	**91.9**	**97.4**	**99.1**	**95.7**	**98.7**	**95.7**

* Not detected or below limit of quantification
n.p.: Not performed

Table A7.2.1_8: Pattern of degradation and formation of metabolites in soil II (Senozan). Values in percent of applied radioactivity

^{14}C-Triclosan Soil II (clay loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Parent	A	95.6	75.8	60.8	46.9	28.4	12.3	4.5	3.6	*
	B	92.9	75.8	62.7	47.6	26.1	13.3	6.4	2.7	*
	mean	**94.2**	**75.8**	**61.8**	**47.3**	**27.2**	**12.8**	**5.4**	**3.2**	*
M1 (unknown)	A	2.6	2.3	0.9	*	*	*	*	*	*
	B	4.2	1.6	0.9	*	*	*	*	*	*
	mean	**3.4**	**1.9**	**0.9**	*****	*****	*****	*****	*****	*
M3 (methyl-triclosan)	A	*	*	0.6	4.0	13.9	18.1	24.1	17.8	17.8
	B	*	*	1.6	2.2	16.5	20.1	23.9	21.9	16.8
	mean	*****	*****	**1.1**	**3.1**	**15.2**	**19.1**	**24.0**	**19.9**	**17.3**
Non-Extractables	A	2.0	22.0	37.3	43.6	48.3	61.1	60.0	59.5	60.7
	B	2.1	23.0	34.2	44.5	53.5	58.3	59.3	56.8	60.9
	mean	**2.1**	**22.5**	**35.7**	**44.0**	**50.9**	**59.7**	**59.6**	**58.1**	**60.8**
$^{14}CO_2$	A	n.p.	<0.1	0.9	<0.1	4.4	6.8	9.8	11.6	18.5
	B	n.p.	<0.1	1.3	<0.1	4.1	7.4	9.2	11.6	14.0
	mean	**n.p.**	**<0.1**	**1.1**	**<0.1**	**4.2**	**7.1**	**9.5**	**11.6**	**16.2**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.2
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.1**
TOTAL	**A**	**100.1**	**100.2**	**100.6**	**94.6**	**95.1**	**98.4**	**98.4**	**92.7**	**97.1**
	B	**99.2**	**100.5**	**100.8**	**94.3**	**100.2**	**99.2**	**98.8**	**93.1**	**92.0**

* Not detected or below limit of quantification
n.p.: Not performed

Table A7.2.1_9: Pattern of degradation and formation of metabolites in soil III (Gartenacker). Values in percent of applied radioactivity

^{14}C-Triclosan Soil III (loam) (% applied)	Duplicate	INCUBATION TIME IN DAYS								
		0	1	2	3	7	14	28	61	124
Parent	A	92.4	69.2	52.0	43.4	34.9	11.4	6.1	3.2	1
	B	91.3	68.8	50.9	41.2	26.3	13.5	9.1	4.0	1.2
	mean	**91.9**	**69.0**	**51.5**	**42.3**	**30.6**	**12.5**	**7.6**	**3.6**	0.9
M1 (unknown)	A	4.2	1.1	0.7	*	*	*	*	*	*
	B	2.7	1.0	0.8	*	*	*	*	*	*
	mean	**3.5**	**1.1**	**0.7**	*****	*****	*****	*****	*****	*
M3 (methyl-triclosan)	A	*	*	*	*	8.5	14.1	15.6	9.5	2.8
	B	*	*	1.2	*	3.9	12.8	9.1	6.7	5.5
	mean	*****	*****	**0.6**	*****	**6.2**	**13.4**	**12.4**	**8.1**	**4.2**
Non-Extractables	A	4.3	29.4	44.8	50.2	50.2	69.0	69.1	73.6	78.4
	B	4.2	29.8	45.5	52.1	66.2	67.9	73.6	74.8	73.2
	mean	**4.2**	**29.6**	**45.1**	**51.1**	**58.2**	**68.5**	**71.3**	**74.2**	**75.8**
$^{14}CO_2$	A	n.p.	<0.1	1.1	1.2	2.8	<0.1	6.2	5.4	9.8
	B	n.p.	<0.1	1.2	1.3	3.0	<0.1	5.9	9.8	13.2
	mean	**n.p.**	**<0.1**	**1.1**	**1.2**	**2.9**	**<0.1**	**6.0**	**7.6**	**11.5**
Organic Volatiles	A	n.p.	<0.1	<0.1	1.1**	<0.1	<0.1	<0.1	<0.1	0.7**
	B	n.p.	<0.1	<0.1	1.2**	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**1.1****	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**0.4****
T O T A L	**A**	**100.9**	**99.9**	**98.6**	**95.8**	**96.5**	**94.7**	**97.0**	**91.7**	**100.5**
	B	**98.2**	**99.6**	**99.5**	**95.8**	**99.4**	**94.2**	**97.8**	**95.4**	**99.9**

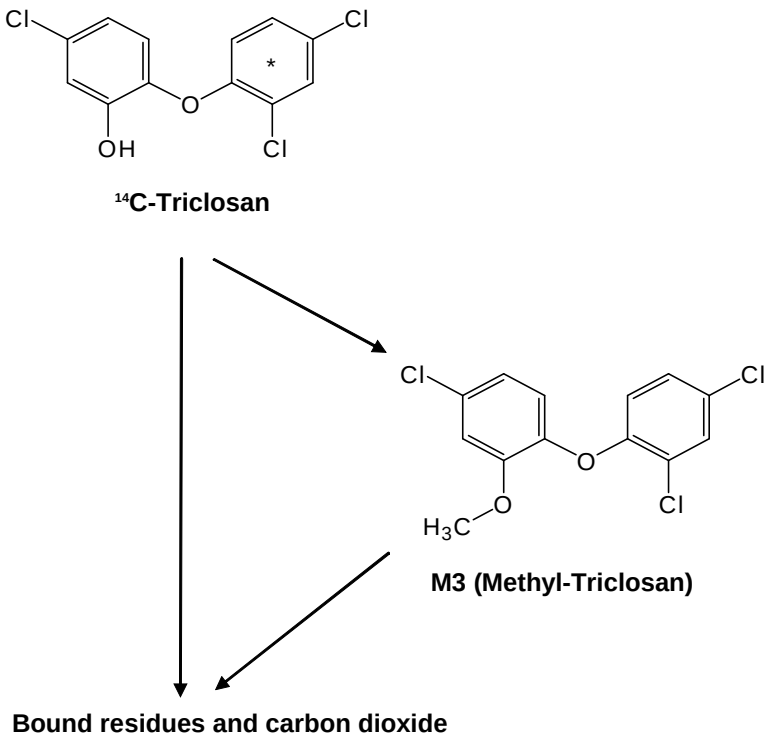
* Not detected or below limit of quantification
** This was probably only due to a contamination of the trapping flask since no continuous increase was observed.
n.p.: Not performed

Table A7.2.1_10: Pattern of degradation and formation of metabolites in soil I (Speyer 5M) at 10 °C. Values in percent of applied radioactivity

^{14}C-Triclosan Soil I at 10°C (% applied)	Duplicate	INCUBATION TIME IN DAYS						
		0	3	7	14	28	61	124
Parent	A	92.2	72.8	53.7	39.9	29.9	13.9	11.9
	B	94.7	72.5	61.5	39.5	32.8	26.8	23.0
	mean	**93.4**	**72.7**	**57.6**	**39.7**	**31.3**	**20.3**	**17.4**
M1 (unknown)	A	3.6	1.5	0.8	*	*	*	*
	B	4.0	1.4	0.8	*	*	*	*
	mean	**3.8**	**1.5**	**0.8**	*****	*****	*****	*
M2 (unknown)	A	1.2	*	*	*	*	*	*
	B	*	*	*	*	*	*	*
	mean	**0.6**	*****	*****	*****	*****	*****	*
M3 (methyl-triclosan)	A	*	*	1.8	4.4	7.9	16.5	15.4
	B	*	*	1.9	3.6	9.8	12.5	11.3
	mean	*****	*****	**1.9**	**4.0**	**8.8**	**14.5**	**13.3**
Non-Extractables	A	1.9	23.2	40.9	53.9	54.9	61.3	63.7
	B	1.9	17.6	34.8	52.9	51.9	52.8	55.6
	mean	**1.9**	**20.4**	**37.8**	**53.4**	**53.4**	**57.1**	**59.6**
$^{14}CO_2$	A	n.p.	<0.1	1.2	2.4	3.2	3.6	4.9
	B	n.p.	0.1	1.1	2.4	2.6	3.8	5.3
	mean	**n.p.**	**<0.1**	**1.2**	**2.4**	**2.9**	**3.7**	**5.1**
Organic Volatiles	A	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	B	n.p.	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
	mean	**n.p.**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**	**<0.1**
T O T A L	**A**	**98.9**	**97.6**	**98.4**	**100.6**	**95.9**	**95.4**	**95.8**
	B	**100.6**	**91.7**	**100.1**	**98.5**	**97.1**	**95.8**	**95.3**

* Not detected or below limit of quantification
n.p.: Not performed

Figure 1: Pathway of degradation of Triclosan in soil



Section A7.2.1 Aerobic degradation in soil (02)

Annex Point: IIIA XII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1994a): Triclosan - Determination of aerobic biodegradation in soils. [redacted] Report #: [redacted] Study #: 1[redacted] Date: 12.04.1994.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	U.S. FDA Technical Assistance Document, Section 3.12	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes; deviations are not expected to alter the results of the study	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Radiolabeled test substance: ^{14}C-labelled Triclosan	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	Specific radioactivity: 15 µCi/mg	
3.1.3	Purity	Radiochemical purity: 94.8%	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	<u>HPLC analysis:</u> The concentration of ^{14}C-Triclosan in each soil was determined on day 0 in the treated soil remaining after distribution of the specified aliquots to the test vessels, and biweekly thereafter, in triplicate samples taken from the 2,000 mL flasks containing 1,000 g of treated soil. Soil samples were extracted using an organic solvent (Tetrahydrofuran) and the extracts were analyzed by high performance liquid chromatography with radiometric detection (HPLC-RAM). Instrumental conditions: Instrument: Waters solvent pump Model 510 equipped with a Waters Model 710B autosampler and Backman Model 171 Radio Isotope Detector Column: Metachem Nucleosil C18 (10 µm), 250 mm (length) x 4.6 mm (ID) Mobile phase: 80% acetonitrile; 20% reagent water Flow rate: 1.0 mL/min Injection volume: 100 µL The radioactivity remaining bound to the extracted soil was quantified by	

Section A7.2.1 Aerobic degradation in soil (02)

Annex Point: IIIA XII 1.1

		LSC to determine extraction efficiency and sample mass balance. On day 64, samples from the 250-mL flasks were also extracted and analysed. Radiometric analysis: On day 0, three aliquots of each ^{14}C-Triclosan treated soil and the resultant $^{14}CO_2$ trapped for LSC counting (Beckman 5000 TD or Packard 1600 CA liquid scintillation counter calibrated with factory prepared standards) were combusted in a Packard Model 306 Sample Oxidizer. The procedure was repeated on day 64 using five aliquots taken directly from the mineralization test flasks for both ^{14}C-Triclosan and ^{14}C-glucose. The soil remaining in the glucose test flasks following removal of the five aliquots was extracted using reagent water, sonicated for 2 minutes and aliquots of the extract were subjected to LSC counting. The remaining extracted soil was air dried, then five additional aliquots were combusted and the amount of radioactivity quantified. The day 0 samples served to verify dosing; the day 64 samples were used to determine radioactive material balance. Metabolite Identification: Pooled extracts of the samples collected on day 64 were analysed by liquid chromatography coupled with mass spectrometry (LC-MS) to determine the identity of the principal metabolite formed in all three soils. The spectra were compared to those supplied by an electronic MS library to verify the probable identity of the compound. Instrumental conditions: Instrument: Hewlett-Packard Model 1050 HPLC solvent pump, Hewlett-Packard Model 1050 HPLC autosampler, Hewlett-Packard Model 5989A MS engine with a particle-beam interface (LC/MS) Column: Phenomenex Ultremex C18 (5 µm), 250 mm (length) x 2.0 mm (ID) Mobile phase: 90% acetonitrile; 10% reagent water Flow rate: 0.4 mL/min Injection volume: 2-25 µL Detection: MS (El, scan)
3.2	**Reference substance**	Glucose (dextrose), Lot #: 916671A, Stock concentrations: 100% purity ^{14}C-Glucose, Lot #: 2482-283, specific activity 2.4 mCi/mmole, radiochemical purity 99.0%
3.2.1	Method of analysis for reference substance	Quantification of radiolabeled glucose via LSC; no qualitative analysis
3.3	**Soil types**	Three soil types were used, see table A7.2.1_1
3.4	**Testing procedure**	
3.4.1	Test system	Determination of the biodegradation potential of Triclosan in soils by the carbon dioxide evolution method. The amounts of ^{14}C-carbon dioxide (CO_2) and ^{14}C-volatile products released upon biodegradation of ^{14}C-Triclosan and ^{14}C-glucose in soils were determined for 64 days.
3.4.2	Test solution and Test conditions	Each test system for the collection of evolved radiolabeled gases consisted of a 250 mL glass Erlenmeyer flask fitted with a silicone rubber stopper into which glass tubing was inserted to provide inlet and outlet ports for air exchange. The inlet port was connected to a tube filled with Ascarite absorbent to remove atmospheric CO_2 before air entered

Section A7.2.1 Aerobic degradation in soil (02)

Annex Point: IIIA XII 1.1

the flask. A series of trapping vials was connected to the outlet port: a backflow trap, two volatile traps, a second backflow trap and two CO_2 traps.

One additional test system, consisting of a 2,000 mL glass Erlenmeyer flask fitted with a silicone rubber stopper as described above, was established for each soil type. This flask was not connected to the trapping system; rather, it provided soil for the chemical-specific analysis for Triclosan.

All flasks were incubated aerobically in the dark in an environmental chamber maintained at 22 ± 3 °C during the test period. x

Test article:
A test concentration in the Arkansas silt loam, Kansas loam and Wisconsin sandy loam (sieved < 2 mm) of 200 µg ^{14}C-Triclosan/kg was produced.
50 g (dry weight) aliquots of the treated soils were added to the 250 mL flasks. Three flasks were established for each soil type.
1000 g of treated soil was added to the 2,000 mL flasks. One flask was established for each soil type.

Glucose:
Flasks intended to hold glucose-fortified soil received radiolabelled glucose stock and non-radiolabeled glucose stock to provide 25 mg glucose/50 g dry soil (500 mg/kg), equivalent to 10 mg C/50 g dry soil. Three glucose-amended flasks were established for each soil type.

Test article + Glucose:

In addition, all soils were amended with activated sludge obtained from an industrial sewage treatment plant receiving Triclosan in the waste stream, in San Juan, Puerto Rico to further decrease the potential for toxicity and to increase the likelihood that biodegradation would occur. Hydration level of 70% of field moisture capacity was maintained during the study (remoisturization on a weekly basis).

Each 250 mL flask was aerated under negative pressure for one hour each day. Trapping vials were changed with vials containing fresh solution and the vials removed were analysed by liquid scintillation counting (LSC) daily for the first 10 days an then at least twice weekly for the remainder of the test.
The 2,000 mL flasks were allowed passive gas exchange with the atmosphere via the two glass tubes inserted in the stopper.

4 RESULTS

4.1 Aerobic soil metabolism

See tables A7.2.1_2 to A7.2.1_4

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Determination of the biodegradation potential of Triclosan in soils by the carbon dioxide evolution method according to the guideline (see point 2.1).

5.2 Results and discussion

Section A7.2.1 Aerobic degradation in soil (02)

Annex Point: IIIA XII 1.1

5.2.1	DT50 values	Arkansas silt loam: Half-life ($t_{1/2}$) = 35.2 days Slope (k) = -0.020 Correlation coefficient (r^2) = 0.900 Kansas loam: Half-life ($t_{1/2}$) = 29.1 days Slope (k) = -0.024 Correlation coefficient (r^2) = 0.958 Wisconsin sandy loam: Half-life ($t_{1/2}$) = 17.4 days Slope (k) = -0.040 Correlation coefficient (r^2) = 0.890	
5.2.2	Degradation products (% of a.s.)	Chemical-specific analysis by HPLC-RAM indicated that primary biodegradation of Triclosan occurred, resulting in the loss of 77.3%, 79.3% and 93.1% Triclosan initially applied to the Arkansas, Kansas and Wisconsin soils, respectively. The principal metabolite formed in all three soils was identified using liquid chromatography/mass spectroscopy. Comparison of the mass spectrum of the metabolite to library references resulted in identification of methoxytriclosan. Material balance (3 replicates) for Triclosan at completion of testing (Day 64) ranged from 93-99% for the Arkansas soil, 87-93% for the Kansas soil and 101-106% for the Wisconsin soil. Material balance (3 replicates) for glucose at completion of testing ranged from 90-97% for the Arkansas soil, 68-80% for the Kansas soil and 87-96% for the Wisconsin soil.	x
5.2.3	Bound residues	The percent non-extractable residue in the three soils ranged from 12.6% to 80.5% and generally increased with time. Because the extraction method was developed specifically to remove Triclosan from the soils, it is assumed that all the Triclosan is indeed extracted from each sample and that the remaining radioactivity represents non-extractable metabolites. Because of the highly sorptive nature of these degradates, neither their chromatographic characteristics (i.e., more or less polar) nor their identity could be determined in this study.	
5.2.4	CO_2 formation	The mean cumulative radiolabeled carbon dioxide ($^{14}CO_2$) evolution rates of soils fortified with ^{14}C-Triclosan were 20.1% for Arkansas silt loam, 11.9% for Kansas loam and 13.6% for Wisconsin sandy loam after 64 days of testing. Production of volatile products did not exceed a mean cumulative percent of 0.0% in any soil type and thus was not considered significant. Thus, data for cumulative volatile products production are not presented. Testing performed with a reference material, ^{14}C-glucose, in the same soil types resulted in mean cumulative radiolabeled carbon dioxide evolution rates of 50.5% for Arkansas silt loam, 42.8% for Kansas loam and 42.8% for Wisconsin sandy loam after 64 days of testing.	
5.3	**Conclusion**	Half-life of triclosan was calculated as 35.2 days in Arkansas silt loam, 29.1 days in Kansas loam and 17.4 days in Wisconsin sandy loam. The reasonably high correlation coefficients (r^2 = 0.89-0.96) indicate that the linear model adequately describes the primary biodegradation kinetics of	

Section A7.2.1 Aerobic degradation in soil (02)

Annex Point: IIIA XII 1.1

		Triclosan in aerobic soils. The results of this study indicated that Triclosan undergoes both primary biodegradation and complete mineralization under the conditions tested.	x
5.3.1	Reliability	2-3	
5.3.2	Deficiencies	Methoxytriclosan (metabolite of triclosan) was not quantified; no identification and quantification of further metabolites; nature of bound residues not clarified.	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	August 2011
Materials and Methods	3.4.2: Exchange “at 22 ± 3 °C” by “23-27.5 ºC”.
Results and discussion	Agree with applicant´s version.
Conclusion	5.2.2: Exchange term “methoxytriclosan” by “methyltriclosan”. 5.3: *Addition* :”to a lesser content” after term “complete mineralization”. 5.3.2: Exchange term “methoxytriclosan” by “methyltriclosan”.
Reliability	2
Acceptability	Acceptable for environmental risk assessment with amendments given above.

Remarks	Predates OECD Guideline 307 (2002), Aerobic and Anaerobic Transformation in Soil. The soils used in this study are American soils. The soils were amended with activated sludge from an industrial STP (not foreseen by U.S. FDA Technical Assistance Document, Section 3.12 but by OECD Guideline 307 when the major route of entry in soil is through sewage sludge). The deviations from the guideline are not described: The temperature was 23-27.5 °C during the experiment. No correction was made for the radiopurity of the test substance. No data on background radioactivity are provided in the report. The original protocol states the use of an alternative substrate (i.e., the reference chemical) to reach a combined substrate concentration of 10 mg C/50 g of soil dry weight in case the test item is inhibitory to microorganisms: Instead, a low test item concentration of 200 µg/kg was used.

Table A7.2.1_1: Classification and physico-chemical properties of the soils used

Soil	Arkansas	Kansas	Wisconsin
Texture	Silt loam	Loam	Sandy Loam
pH	5.9	6.5	7.5
Calcium [lb/ac]	1700	5300	4000
Magnesium [lb/ac]	260	870	900
Organic matter [%]	1.6	3.7	5.7
Total Nitrogen [%]	0.094	0.169	0.260
CEC [meq / 100 g soil]	9.8	22.4	17.1
Particle size:			
Clay [%]	35	31	59
Silt [%]	51	43	29
Sand [%]	14	26	12
Field moisture capacity [% moisture at 1/3 bar]	29.7	35.3	28.1

Table A7.2.1_2: Mean (n = 3) cumulative percent of $^{14}CO_2$ evolved from the test soils fortified with ^{14}C-Triclosan and ^{14}C-glucose

Incubation time (days)	Triclosan $^{14}CO_2$ [%]			Glucose $^{14}CO_2$ [%]		
	Arkansas Soil	Kansas Soil	Wisconsin Soil	Arkansas Soil	Kansas Soil	Wisconsin Soil
1	1.0	0.5	0.6	22.4	20.4	20.7
2	2.2	1.0	1.3	27.2	27.2	23.7
3	3.3	1.5	2.0	31.8	28.9	25.4
4	4.2	2.0	2.6	33.6	30.2	26.6
5	5.0	2.4	3.1	35.0	31.1	27.5
6	5.8	2.8	3.5	36.4	31.8	28.2
7	6.5	3.1	3.9	37.6	32.4	28.3
8	7.2	3.5	4.3	38.6	33.1	29.6
9	7.7	3.8	4.6	39.4	33.7	30.3
10	8.4	4.2	4.9	40.3	34.2	31.0
12	9.4	4.8	5.5	41.4	34.9	31.8
15	10.7	5.6	6.6	42.8	35.9	33.9
19	12.0	6.6	7.4	44.0	36.9	35.1
22	13.1	7.3	8.1	44.8	37.5	35.9
26	14.3	7.9	9.0	45.8	38.5	37.3
29	15.0	8.3	9.5	46.3	39.0	37.9
33	15.8	8.8	10.1	46.8	39.3	38.5
36	16.3	9.2	10.5	47.1	39.7	39.1
40	16.5	9.6	10.9	47.6	39.9	39.7
43	17.5	10.0	11.6	47.9	40.2	40.2
47	18.3	10.4	12.1	48.6	40.5	41.0
54	19.1	11.0	12.7	49.6	41.9	41.7
61	19.8	11.6	13.3	50.2	42.5	42.4
64	20.1	11.9	13.6	50.5	42.8	42.8

Table A7.2.1_3: Concentrations of Triclosan and glucose (n = 3, each) in the test soils as determined by HPLC-RAM

Incubation time (days)	Triclosan								
	Arkansas Soil			Kansas Soil			Wisconsin Soil		
	RA % Extrac.	RA % Parent*	RA % Bound	RA % Extract.	RA % Parent*	RA % Bound	RA % Extrac.	RA % Parent*	RA % Bound
0	63.8	79.9	35.2	83.9	94.3	13.9	77.3	94.0	38.5
	64.4	93.8	49.4	90.5	94.7	17.9	76.5	95.7	42.6
	63.7	93.2	58.1	92.0	91.2	12.6	95.3	96.1	53.5
14[a]	41.9	91.4	61.5	61.6	82.9	26.0	46.1	50.5	74.2
	42.4	100.0	69.6	62.3	81.1	29.5	49.9	56.2	78.5
28	45.8	49.8	64.9	71.1	64.2	30.2	47.9	22.6	51.3
	48.4	86.8	77.1	75.1	65.2	34.6	46.4	24.3	59.7
	52.2	84.9	61.2	74.4	69.5	34.4	42.0	28.2	68.9
42	37.2	78.5	75.2	57.5	46.4	35.0	39.2	19.5	70.2
	41.6	71.5	80.5	58.6	43.4	33.6	39.4	19.6	62.9
	37.8	74.5	70.5	58.3	39.6	43.2	33.9	14.8	66.9
56	40.0	63.6	37.8	53.5	36.2	28.3	46.7	11.6	34.0
	37.1	60.6	41.3	64.3	35.0	28.0	45.4	10.2	37.5
	38.3	58.3	38.4	66.2	38.9	27.5	43.3	20.7	34.1
64	26.5	39.5	42.7	47.6	39.7	34.4	35.5	14.7	40.6
	27.3	47.2	42.9	48.2	32.7	26.5	37.0	18.4	44.4
	31.5	48.6	41.6	49.6	34.1	27.5	32.8	13.1	45.9
64M[b]	16.8	34.0	61.7	39.6	35.0	33.8	29.6	12.0	56.1
	13.0	21.6	52.8	43.4	27.9	36.3	32.6	11.7	53.4
	13.4	NA[c]	64.6	40.6	29.5	42.9	30.0	8.6	50.5
Control samples									
0 & 14[d]	103	92.9	14.7	103	95.3	17.1	116	94.9	20.7
28	98.7	91.5	8.1	97.8	91.4	10.2	105	88.9	13.1
42	103	100	8.6	102	100	7.9	101	95.0	9.9
56	138	96.5	3.77	134	100	6.15	133	96.1	4.09
64	108	94.7	6.55	104	92.4	3.74	101	100	5.81

[*] percent of extractable amount
[a] only 2 replicates
[b] Day 64 mineralization flasks; not included in the half-life calculation
[c] Not applicable - extract lost during preparation
[d] Test samples for days 0 and 14 were analyzed on the same day, using a single set of QC samples

Table A7.2.1_4: Radioactivity material balance of triclosan and glucose (as percent) in the test soils (day 64)

	Triclosan			Glucose		
	Arkansas soil	Kansas soil	Wisconsin soil	Arkansas soil	Kansas soil	Wisconsin soil
Replicate 1	96.6%	92.5%	106%	90.8%	4.2%	91.4%
Replicate 2	92.7%	88.8%	101%	96.9%	67.6%	95.7%
Replicate 3	99.1%	86.5%	101%	90.4%	79.8%	86.7%
Mean	96.1%	89.3%	102%	92.7%	73.7%*	91.3%

* Replicate 1 is not included in the mean due to low mass balance. A dosing error is suspected.

Section 7.2.3.1 **Annex Point IIIA 12.2.2**	**Adsorption and desorption in accordance with EC C18 (or OECD 106) and where relevant adsorption and desorption for metabolites**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [...]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	A reliable screening test using High Performance Liquid Chromatography (HPLC) was done for DCPP. Therefore, a batch equilibrium study in accordance with EC C19 (or OECD 106) is not considered as being relevant.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	December 2010	
Evaluation of applicant's justification	Agree with applicant’s version.	
Conclusion	Agree with applicant’s version.	
Remarks	-	

Section A7.3.1 **Phototransformation in air**

Annex Point: IIIA, VII 5 **(estimation method)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2007a): DCPP. Calculation of indirect photodegradation. [redacted] Report No. [redacted] Date: 2007-02-02.	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	No; Calculation based on QSAR	
2.2	**GLP**	Not applicable since the degradation behaviour of DCPP in air was calculated.	
2.3	**Deviations**	Not applicable since the degradation behaviour of DCPP in air was calculated.	
		3 MATERIALS AND METHODS	
		The half-life of DCPP in air due to indirect photodegradation, i.e. oxidation of organic chemicals with photochemically produced hydroxyl radicals, was calculated using the software programme AOPWIN (v. 1.91, 2000, US-EPA) based upon QSAR methods developed by Dr. Roger Atkinson and co-workers. AOPWIN requires the CAS No. or a chemical structure to make these predictions. Structures are entered into AOPWIN by SMILES (Simplified Molecular Input Line Entry System) notations. The estimation for DCPP was carried out with respect to the OH radical reaction, using a 24-hours-day with 5 x 10^5 OH radicals/cm^3.	
		4 RESULTS	
		The half-life of DCPP in the troposphere is calculated to be 19.701 hours with a degradation rate of 19.5447E^{-12} cm^3*$molecule^{-1}$*s^{-1}. This corresponds to a chemical lifetime in air of 0.821 days.	
		5 APPLICANTS SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The half-life of DCPP in air due to indirect photodegradation, i.e. oxidation of organic chemicals with photochemically produced hydroxyl radicals, was calculated using the software programme AOPWIN (v. 1.91, 2000, US-EPA) based upon QSAR methods developed by Dr. Roger Atkinson and co-workers.	

Section A7.3.1 Phototransformation in air
Annex Point: IIIA, VII 5 (estimation method)

5.2	**Results and discussion**	The half-life of DCPP in the troposphere is calculated to be 19.701 hours with a degradation rate of $19.5447E^{-12}$ $cm^3*molecule^{-1}*s^{-1}$. This corresponds to a chemical lifetime in air of 0.821 days.
5.3	**Conclusion**	Due to the short chemical lifetime of DCPP in air it is not to be expected that the compound will accumulate in air or be transported in the gaseous phase over long distances. Hence, air will not be an environmental compartment of concern.
5.3.1	Reliability	2
5.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2010
Materials and Methods	3: Addition The estimation for DCPP was carried out with respect to the OH radical reaction, using a 24-hours-day with 5 x 10^5 OH radicals/cm^3. An excerpt from the AOPWIN protocol is presented below. SMILES : CLc2cc(O)c(Oc1ccc(CL)cc1)cc2 MOL FOR: C12 H8 CL2 O2 MOL WT : 255.10 ------ SUMMARY (AOP v1.92): HYDROXYL RADICALS (25 deg C) ------ Hydrogen Abstraction = 0.0000 E-12 cm3/molecule-sec Reaction with N, S and -OH = 0.1400 E-12 cm3/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec **Addition to Aromatic Rings = 19.4047 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 19.5447 E-12 cm3/molecule-sec HALF-LIFE = 0.821 Days (24-hr day; 0.5E6 OH/cm3) HALF-LIFE = 19.701 Hrs ** Designates Estimation(s) Using ASSUMED Value(s)
Results and discussion	Addition: The half-life of DCPP in the troposphere is calculated to be 19.701 hours corresponding to 0.821 days (24 h day, 5x10^5 radicals/cm^3). Based on this value a $kdeg_{air}$-value of 0.84 d^{-1} was derived. $kdeg_{air}$ = 0.84 d^{-1} with k_{OH}: specific degradation rate constant with OH-radicals [cm^3x$molec.^{-1}$xs^{-1}]- 19.5447 x 10^{-12} cm^3x$molec.^{-1}$xs^{-1} (see AOPWIN protocol excerpt given above) c_{OH} = 5*10^5 moleculesxcm^{-3} acc. to TGD $kdeg_{air}$ (pseudo 1st order rate const. for degr. in air) = k_{OH} • c_{OH} • 24 • 3600 [d^{-1}]
Conclusion	5.3: Substitution: The half-life of DCPP in the troposphere is calculated to be 19.701 hours corresponding to 0.821 days (24 h day, 5x10^5 radicals/cm^3). Based on this value a $kdeg_{air}$-value of 0.84 d^{-1} was derived.
Reliability	1
Acceptability	Acceptable
Remarks	-

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Danio rerio* (formerly *Brachydanio rerio*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]B. (1999b): Acute toxicity of [redacted] to zebra fish (*Brachydanio rerio*) in a 96-hour static test. [redacted] Report No. [redacted] 1999-04-06 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Directive 92/69/EEC, C.1 and OECD No. 203 (1992)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](DCPP)	
3.1.1	Lot/Batch number	Batch No.[redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	>99%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Stability in water: >1 year (estimated half-life at 25 °C; determined in RCC Project 712260) Solubility in water: 19.5 mg/L (determined in RCC Project 712012)	x
3.1.6	Method of analysis	Yes, HPLC analysis and UV/VIS-detection, method is attached to the report	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test medium of the highest test item concentration of nominal 10 mg/L was prepared dissolving 30 and 31 mg of the test item completely in 3 L test water each by 20 minutes ultrasonic treatment and 72 hours intense stirring. The stirring period was chosen to make sure that the test item was completely dissolved and was based on the results of pre-tests (without GLP) where a solubility of about 10-20 mg/L and a stability of at least 96 hours were determined. After stirring, the two solutions were filled in one aquarium and intensively mixed. Adequate volumes of this test medium with a nominal concentration of 10 mg/L were added to test water to prepare the test media of the lower nominal concentrations of 4.6, 2.2, 1.0 and 0.46 mg/L. See also table A7_4_1_1-1	
3.3	**Reference**	No	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Danio rerio* (formerly *Brachydanio rerio*)

	substance		
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_1-2	
3.4.2	Test organisms	See table A7_4_1_1-3	
3.4.3	Test system	See table A7_4_1_1-4	x
3.4.4	Test conditions	See table A7_4_1_1-5	
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and symptoms of intoxication	
3.4.7	Sampling	The test fish were observed after approximately 2, 24, 48, 72 and 96 hours test duration for symptoms of intoxication and mortality. Dead fish were removed at least once daily and discarded. The pH-values, the oxygen concentrations and the water temperature of the test media and the control were measured at the start of the test and once every day during the test. The appearance of the test media was recorded at least daily.	
3.4.8	Monitoring of TS concentration	For the analytical measurements of the test item concentrations duplicate samples from the freshly prepared test media of all test concentrations and the control were taken at the start of the test (Day 0). For the determination of the maintenance of the test item concentrations during the test period, duplicate samples were taken out of all test media and the control after two days and at the end of the test (Day 4), respectively when all fish were dead in one concentration. All samples were taken from the approximate center of the aquaria without mixing of the test media, and were deep-frozen (at about -20 °C) immediately after sampling. The concentrations of the test item DCPP were analyzed in the duplicate test media samples from the test concentrations of nominal 0.46 to 4.6 mg/L from Day 0, of nominal 0.46 and 1.0 mg/L from Day 2 and Day 4 and of nominal 2.2 mg/L from Day 1 since all fish were dead in this concentration at this time. The samples from the test concentration of nominal 10 mg/L were not analyzed, since the same high toxic effect was determined in the next lower analyzed samples of nominal 4.6 mg/L. The test concentration of nominal 10 mg/L was therefore not considered as being a relevant part of the concentration-effect relationship. From the control samples only one of the duplicate samples was analyzed from Day 0, Day 2 and Day 4.	
3.4.9	Statistics	The LC_{50} and the 95% confidence interval at the observation dates were calculated as far as possible by Probit Analysis. The biological results of the test concentration of nominal 10 mg/L were not taken into account at the calculation. The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the raw data.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Danio rerio* (formerly *Brachydanio rerio*)

		The LC_{50} at the observation intervals after 2 hours could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead the LC_{50}-value was determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% mortality, and the 95% confidence intervals for the LC_{50} as the test concentrations with 0% and 100% mortality.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	The test concentrations were based on the results of a range-finding test (without GLP) and the results of pre-experiments to the solubility of the test item. Nominal concentrations: 0.46, 1.0, 2.2, 4.6, and 10 mg/L, and a control.	
4.2.2	Actual concentrations of test substance	The analytical determined mean test item concentrations in the test media varied in the range of 37 to 115% of the nominal values during the whole test period. At the start the mean measured test item concentrations ranged from 110 to 115% of the nominal values. After 48 hours of incubation 37-41% of nominal were found in the low-level samples while after 96 hours of incubation 69-70% of nominal were found in these samples. As the test item is hydrolytically stable and considerably soluble in fat, adsorption and subsequent desorption and re-solution may be a reason for the observed fluctuations. See table A7_4_1_1-6.	
4.2.3	Effect data (Mortality)	See table A7_4_1_1-7	x
4.2.4	Concentration / response curve	A concentration/response curve is not given in the report	
4.2.5	Other effects	See table A7_4_1_1-7 for detailed description of observed symptoms of intoxication.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing	There were neither mortalities nor symptoms of intoxication in the dilution water control.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Danio rerio* (formerly *Brachydanio rerio*)

	adverse effects		
4.3.2	Nature of adverse effects	-	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute toxicity of the test item DCPP to zebra fish (*Brachydanio rerio*) was determined in a 96-hour static test according to the Commission Directive 92/69/EEC, Annex Part C.1, and the OECD Guideline for Testing of Chemicals No. 203. The nominal test concentrations were 0.46, 1.0, 2.2, 4.6, and 10 mg/L, and a control.	
5.2	**Results and discussion**	During the test period of 96 hours a decrease of test item concentration in the analyzed test media was determined. The test item is hydrolytically stable and is considerably soluble in fat. Therefore, e.g. adsorption and subsequent desorption and re-solution may be a reason for the observed fluctuations. All reported results are related to total mean measured concentrations of the test item which were in the range of 74-112% of the nominal values. At the total mean measured test item concentration of 0.34 mg/L all fish survived until the end of the test and no symptoms of intoxication were observed. At the next higher test concentrations of total mean measured 0.74 and 2.2 mg/L all test fish showed one or several intoxication symptoms. At the end of the test four of the seven test fish had died at the test concentration of 0.74 mg/L. The fish in the test concentration of 2.2 mg/L had died within one day. At the two highest test concentrations all fish were dead already about 2 hours after introduction into the test media.	
5.2.1	96h-LC_0	0.34 mg a.s./L (NOEC)	
5.2.2	96h-LC_{50}	0.70 mg a.s./L (95% C.I. = 0.54 and 0.92 mg a.s./L)	
5.2.3	96h-LC_{100}	2.2 mg a.s./L	
5.3	**Conclusion**	The test is considered as valid. The validity criteria are summarised in table A7_4_1_1-8.	
5.3.1	Other Conclusions	-	
5.3.2	Reliability	1	x
5.3.3	Deficiencies	-	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	December 2010
Materials and Methods	3.1.5 *Addition*: Vapour pressure: 1.2 x 10^{-6} (at 25°C; determined in RCC Project 711990)
Results and discussion	4.2.3: ad Table A7_4_1_1-7: *Deletion of phrase* " , and observed symptoms of intoxication" in the heading of table A7_4_1_1-7
Conclusion	5.3.2: Change to 2 5.3.3: *Addition:* Static test conditions were suboptimal as the test concentration could not be maintained $\geq$ 80% of the nominal concentration. No reasons for choice of the test procedure used are provided. No graph of the concentration/percentage response curve is provided.
Reliability	2 as the study is a GLP study conducted according to an internationally accepted Guideline but the static test conditions were suboptimal as the test concentration could not be maintained $\geq$ 80% of the nominal concentration. For a substance with high adsorption properties a semi-static or flow-through test system would have been preferable.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No solvent used
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Reconstituted test water: analytical grade salts were dissolved in deionized water to obtain the following nominal concentrations: $CaCl_2$ x $2H_2O$: 2.0 mmol/ L (= 294 mg/L) $MgSO_4$ x $7H_2O$: 0.5 mmol/ L (= 123 mg/L) $NaHCO_3$: 0.75 mmol/ L (= 65 mg/L) KCl: 0.075 mmol/ L (= 5.8 mg/L) Ratio of Ca:Mg = 4:1 (based on molarity) Na:K = 10:1 (based on molarity)
Alkalinity	0.8 mmol/L
Hardness	2.5 mmol/L (= 250 mg/L) as $CaCO_3$
pH	Measured during the test (see table A7_4_1_1-5)
Oxygen content	The test water was aerated until oxygen saturation was reached.
Conductance	No data
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Zebra fish (*Brachydanio rerio*)
Source	[redacted]
Wild caught	No
Age/size	Mean wet weight: 0.2 ± 0.05 g Mean length: 2.6 ± 0.1 cm
Kind of food	During holding and acclimation until one day before the start of the test the fish were fed with a commercial fish diet (TETRA MIN Hauptfutter, supplied by TETRA-Werke, D-49304 Melle, Germany).
Amount of food	*Ad libitum*
Feeding frequency	Not specified
Pretreatment	In accordance with the test guidelines the fish were held in the laboratories of RCC Ltd without any medication for more than five weeks prior to the test start. They were acclimated for one week prior to the test start to the test water and temperature.
Feeding of animals during test	Fish were not fed one day before and during the study.

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static regime
Renewal of test solution	None
Volume of test vessels	Glass aquarium containing 3 L test medium
Volume/animal	3 L/7 animals
Number of animals/vessel	7
Number of vessels/ concentration	One aquarium
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	21-22 °C
Dissolved oxygen	> 8.3 mg/L (> 60% saturation)
pH	7.7 – 8.1
Adjustment of pH	No
Aeration of dilution water	The test medium and the test water in the control were slightly aerated during the test period.
Intensity of irradiation	Not stated
Photoperiod	16 hours light and 8 hours dark

Table A7_4_1_1-6: Mean measured concentrations

Nominal test concentration	Total mean measured test item concentration (average over all measurements per test concentration)	
0.46 mg/L	0.34 mg/L	(74% of nominal)
1.0 mg/L	0.74 mg/L	(74% of nominal)
2.2 mg/L	2.2 mg/L	(101% of nominal)
4.6 mg/L	5.1 mg/L	(112% of nominal)
10.0 mg/L	not analysed	not applicable

Table A7_4_1_1-7: Mortality and observed symptoms of intoxication (number of affected fish/ number of dead fish, and observed symptoms of intoxication)

Nominal concentration (mg/L)	Observation time				
	2 hours	24 hours	48 hours	72 hours	96 hours
Control	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
0.46 (0.34)#	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0
1.0 (0.74)#	6 / 0 AP, BO, TS	7 / 1 AP, OB, TS	7 / 3 AP, OB, SR, TS	4 / 4	4 / 4
2.2 (2.2)#	7 / 0 AP, BO, SR, TS	7 / 7	* / *	* / *	* / *
4.6 (5.1)#	7 / 7	* / *	* / *	* / *	* / *
10 (n.a.)	7 / 7	* / *	* / *	* / *	* / *
LC_{50}	3.3*	0.95	0.78	0.70	0.70
95% C.I.	2.2–5.1	0.43–2.1	0.59–1.1	0.54–0.92	0.54–0.92

* / *: all fish dead

95% C.I.: 95% confidence interval of the LC_{50}

*: this LC_{50}-value was determined as the geometric mean value of the two consecutive test concentrations with 0% and 100% mortality, and the 95% confidence intervals (95% C.I) for the LC_{50} as the test concentrations with 0% and 100% mortality

#: total mean measured concentration (mg/L)

n.a.: not analysed

AK: Strongly extended gills
AP: Apathy
BA: Distended abdomen
BO: Fish mainly at the bottom of the aquarium
GA: Exophthalmus
OB: Fish mainly at the water surface
SA: Mucus secretion
SR: Fish lying on side or back on the bottom
SV: Strong ventilation
TS: Tumbling during swimming
VF: Changed body color

Table A7_4_1_1-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	**Not fulfilled**
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X (effect conc. used)**	

Criteria for poorly soluble test substances	**X**	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted]. Determination of 96h LC50 of [redacted] in an Acute Toxicity Test with the fish Danio rerio – Static Test. [redacted] Report no. [redacted] Date: 2003-03-31 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes - OECD No. 203 (1992) - DIN 38412/15 (1982)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes - Loading not based on fish weight (documentation missing)
		3 MATERIALS AND METHODS
3.1	**Test material**	[redacted](Methyldiclosan, degradation product of Diclosan)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Deviating from specification given in section 2 as follows
3.1.3	Purity	99.7%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-appearance: white powder -water solubility: approx. 0.4 mg/L Determined in a separate study (project 02080201G944) at 0.322 mg/L -homogeneous and stable, not volatile
3.1.6	Method of analysis	Gas chromatography (GC) - solvent: iso-octane - internal standard: hexachlorbenzene - gas chromatograph: GC hp 6890 A, Agilent Technologies - column: Phen_ex 7HG-G002-11, 30 m, 0.25 mm, 0.25 µm - GC method: 02080201G

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

		- linear sector of method: 10-50 mg/L - R of calibration function: 0.9997 - standard deviation of injection: ± 3.6% - recovery rate (water) at 90%: 77±6% - validation report: VB02080201G926
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A super-saturated stock solution of the test item was prepared. This was done by mixing 100 mg/L of the test item with dilution water and shaking vigorously for 24 hours. The resulting solution was filtrated through 0.45 µm filters. The stock solution then was used to prepare the solutions of the concentrations to be tested. See also table A7_4_1_1-1.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Dilution water	Drinking water (see table A7_4_1_1-2)
3.4.2	Test organisms	*Danio rerio* (see table A7_4_1_1-3)
3.4.3	Test system	Static (see table A7_4_1_1-4)
3.4.4	Test conditions	See table A7_4_1_1-5
3.4.5	Duration of the test	96 h
3.4.6	Test parameter	Mortality and behaviour (24 h, 48 h, 72 h, 96 h)
3.4.7	Sampling	No data on sampling technique and sample storage
3.4.8	Monitoring of TS concentration	Yes Sampling of control and test solution at test start and end
3.4.9	Statistics	Not applicable since no mortality occurred
		4 RESULTS
4.1	**Limit Test**	Performed
4.1.1	Concentration	Loading: 100 mg/L; measured: 0.27 mg/L Control: measured: 0 mg/L
4.1.2	Number/ percentage of animals showing adverse effects	0%
4.1.3	Nature of adverse	No mortality or abnormal behaviour observed.

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

	effects		
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Loading: 100 mg/L; measured: 0.27 mg/L Control: measured: 0 mg/L	
4.2.2	Effect data (Mortality)	0%	
4.2.3	Actual concentrations of test substance	measured: - initial: 0.27 mg/L - final (96 h): 0.031 mg/L; recovery based on initial concentration: 11%; - mean measured: 0.15 mg/L - Control: initial (0 h): 0 mg/L: final (96 h): 0.052 mg/L	x
4.2.4	Concentration / response curve	Not applicable	
4.2.5	Other effects	No mortality or abnormal behaviour observed.	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	0%	
4.3.2	Nature of adverse effects	Not applicable	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The acute toxicity of the test item M-DCPP to *Danio rerio* was determined in a 96-hour static test according to the OECD Guideline for Testing of Chemicals No. 203. The test solution was prepared as a super-saturated solution of 100 mg/L (loading). Prior to use the solution was filtered (0.45 µm). Test concentrations were analytically monitored after 0 and 96 h by gas-chromatography. Limit of detection and/or limit of quantification were not given in the report.	
5.2	**Results and discussion**	The analytically determined test item concentration was 0.27 mg/L at test start and 0.031 mg/L at test end. The recovery rate based on the initial concentration was 11%. Because of the chemical properties of M-	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

		DCPP, it was assumed that this was not caused by instability of the test item but by potentially high adsorption and bioaccumulation in fish. In the original report the effect values were based on the initial test concentration. Since no effects were observed which were limited to the start phase, the effect values were re-calculated to the mean concentration (0.15 mg/L).	
		As test item was detected in the control solution at test end, either the control was contaminated or the analytical method was not appropriate to determine exact results. The concentration in the control was even higher than in the corresponding test item solution. No explanation is given in the report.	x
		No mortality or abnormal behaviour of the test animals or other signs of intoxication were observed during the test period of 96 hours. The 96-h LC50 was > 0.15 mg/L (mean measured).	x
5.2.1	LC_0	96-h LC0 ≥ 0.15 mg/L (mean measured)	x
5.2.2	LC_{50}	96-h LC50 > 0.15 mg/L (mean measured)	x
5.2.3	LC_{100}	96-h LC100 > 0.15 mg/L (mean measured)	x
5.3	**Conclusion**	The validity criteria can be considered to be fulfilled with the exception of maintaining the test concentration at a satisfactory level (±80% of initial concentration). As the substance has a high adsorption as well as bioaccumulation potential, the test design was not appropriate at keeping the test item level within acceptable limits.	
5.3.1	Other Conclusions		
5.3.2	Reliability	3	
5.3.3	Deficiencies	Yes	
		- Test concentrations: The test concentrations were not maintained satisfactorily. The recovery is very low: 11% of initial concentration (0 h: 0.27 mg/L; 96 h: 0.031 mg/L).	
		- In addition, the concentration at test end is even lower than the concentration in the control. No explanation is given.	x
		- No analytical limits (LOD/LOQ) are given in the report.	
		- Calculation of effective concentrations: The effective concentrations (96h LC0, 96h LC50, 96h LC100) are based on the initially measured concentrations. Since the fate of the test item is not proven and no effects were observed during the test, the effect values should be based on average concentrations. This is a methodological error.	
		- Loading: The loading is given in fish/L. The guideline specified the loading in g fish/L but there is no documentation about the weight in the study.	x

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA7.1

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2013
Materials and Methods	Agree with the Applicant´s version.
Results and discussion	4.2.3: Replace “mean measured: 0.15 mg/L” by “0.091 mg/L based on geometric mean from measured concentrations at test beginning and end.
Conclusion	5.2. Replace “0.15“ mg/L by “0.091”. 5.2.1: Replace “0.15 mg/L (mean measured)” by “0.091 mg/L based on geometric mean from measured concentrations at test beginning and end”. 5.2.2: Replace “0.15 mg/L (mean measured)” by “0.091 mg/L based on geometric mean from measured concentrations at test beginning and end”. 5.2.3: Replace “0.15 mg/L (mean measured)” by “0.091 mg/L based on geometric mean from measured concentrations at test beginning and end”. 5.3.2: Replace “No explanation is given.” by “The occurrence of Methyl-DCPP in the control was due to an error in the analytical measurement according to the study authors.” 5.3.2: *Addition*: “Nevertheless, using the approximate wet weights according to OECD test guideline 230 (adult fish *Danio rerio*: 0.65 ± 20% for females and 0.4 ± 20% for males) the requirements of the test guideline 203 can be assumed to be fulfilled.”
Reliability	2
Acceptability	Acceptable for environmental risk assessment with amendments given above
Remarks	Although the study has several deficiencies including the decline of the test substance a use seems plausible when applying the geometric mean of the measured concentrations.

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Filtration of stock solution prior to use (0.45 µm)

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	Drinking water
Alkalinity	No data
Hardness	No data
pH	8.28
Oxygen content	Aerated during test
Conductance	246 µS/cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	*Danio rerio*
Source	[redacted]
Wild caught	No
Age/size	Sexually immature young fish (length 2±1 cm)
Kind of food	No data
Amount of food	1-2% of body weight per day
Feeding frequency	three times a day
Pretreatment	Polyethylene aquaria, chlorine-free tap water, change of medium twice a week, photo period 12/12 h, T = 23±1°C
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Static
Renewal of test solution	Not applicable
Volume of test vessels	10 L
Volume/animal	1 L/fish
Number of animals/vessel	7
Number of vessels/ concentration	1
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_1-5: Test conditions

Criteria	Details
Test temperature	23±1 °C
Dissolved oxygen	Control: 8.5 to 9.3 mg/L Test solution: 8.3 to 8.9 mg/L
pH	Control: 7.6 to 8.1 Test solution: 7.6 to 8.1
Adjustment of pH	No
Aeration of dilution water	Yes: 1 bubble/s
Intensity of irradiation	No data (use of fluorescent tubes)
Photoperiod	12/12

Table A7_4_1_1-6: Effect data based on mean measured concentration

	48 h [mg/l]	95 % c.l.	96 h [mg/l]	95 % c.l.
LC_0	≥ 0.15	-	≥ 0.15	-
LC_{50}	> 0.15	-	> 0.15	-
LC_{100}	> 0.15	-	> 0.15	-

Table A7_4_1_1-7: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	Not fullfilled
Mortality of control animals <10%	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	

Concentration of test substance ≥80% of initial concentration during test		**X**

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1999c): Acute toxicity of [redacted] to *Daphnia magna* in a 48-hour immobilization test. [redacted] Report No. [redacted] date: 1999-01-20 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Directive 92/69/EEC, C.2 and OECD No. 202 Part I (1992)	x
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](DCPP)	
3.1.1	Lot/Batch number	Batch No. [redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	>99%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Stability in water: >1 year (estimated half-life at 25 °C; determined in RCC Project 712260) Solubility in water: 19.5 mg/L (determined in RCC Project 712012)	
3.1.6	Method of analysis	HPLC-UV/VIS	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A stock solution of nominal 10 mg/L was prepared by suspending 10 mg of the test item in about 100 mL test water by ultrasonic treatment for 5 minutes first. The suspension was made up to a volume of 1000 mL with test water and dissolved by ultrasonic treatment for 15 minutes and intense stirring for 3 hours. Adequate volumes of the intensively mixed stock solution were added to test water to prepare the following nominal test concentrations: 0.1, 0.22, 0.46, 1.0, and 2.2 mg/L. See also table A7_4_1_2-1.	
3.3	**Reference substance**	None	
3.3.1	Method of analysis for reference substance	-	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	
3.4.2	Test organisms	See table A7_4_1_2-3	x
3.4.3	Test system	See table A7_4_1_2-4	
3.4.4	Test conditions	See table A7_4_1_2-5	x
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobility or mortality	
3.4.7	Sampling	The immobility or mortality of the daphnids was determined by visual controls after 24 and 48 hours of exposure. Those animals not able to swim within 15 seconds after gentle agitation of the test beaker were considered to be immobile. At the start and at the end of the test, the pH-values, the oxygen concentrations and the water temperature were determined in one sample from each test concentration and the control. The appearance of the test media was recorded at the start of the test and after 24 and 48 hours.	
3.4.8	Monitoring of TS concentration	For the analytical measurements of the test item concentrations, one sample from the freshly prepared stock solution and duplicate samples from the freshly prepared test media of all test concentrations and the control were taken just before the start of the test (without daphnids). For the determination of the stability of the test item under the test conditions, respectively the maintenance of the test item concentrations during the test period, sufficient volumes of the freshly prepared test media of all test concentrations and the control were incubated during the test period under the same conditions as in the actual test (but without daphnids). Duplicate samples were taken at the end of the test period. The collecting of samples after 48 hours from the actual test itself was not possible, since the test media volumes in the test were too small for the analytical requirements. All samples were deep-frozen (at about -20 °C) immediately after sampling. The concentrations of the test item DCPP were analyzed in the stock solution sample and in the duplicate test media samples from the test concentrations of nominal 0.22 to 0.46 mg/L and both sampling times (0 and 48 hours). The lowest test item concentration of nominal 0.1 mg/L was not analysed, since it was below the 48-hour NOEC. The highest test item concentrations of nominal 1.0 and 2.2 mg/L were not analysed, since after 48-hours the same toxic effect was determined at the next lower test item concentration of nominal 0.46 mg/L. The test item concentration of nominal 1.0 and 2.2 mg/L therefore were considered as being of no biological relevance for the concentration-effect relationship. From the control samples only one of the duplicate samples was analysed from each of both sampling times.	
3.4.9	Statistics	The 24h- and 48h-EC_{50} could not be calculated by Probit Analysis or Moving Average Interpolation due to the steep concentration-effect relationship. Instead the EC_{50}-value was determined as the geometric mean value of the two consecutive test concentrations with 0% and	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

		100% mortality, and the confidence intervals for the EC_{50} as the test concentrations with 0% and 100% immobility. The NOEC, LOEC, LC_0 and LC_{100} were determined directly from the raw data.
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	The test concentrations were based on the results of a range-finding test and the results of a pre-experiment to the solubility of the test item (without GLP). Nominal concentrations: 0.1, 0.22, 0.46, 1.0, and 2.2 mg/L and control.
4.2.2	Actual concentrations of test substance	The analytical determined mean test item concentrations in the analysed test media varied in the range from 81 to 87% of the nominal values. In the stock solution sample 87% of the nominal concentration was measured. In the test media the test item DCPP was sufficiently stable during the test period of 48 hours. Therefore, all reported biological results are related to the nominal test item concentrations. See table A7_4_1_1-6.
4.2.3	Effect data (Immobilisation)	See table A7_4_1_2-7
4.2.4	Concentration / response curve	A concentration/response curve is not given in the report
4.2.5	Other effects	-
4.3	**Results of controls**	No mortality or abnormal behaviour occurred in the control groups.
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-
		5 APPLICANT'S SUMMARY AND CONCLUSION
5.1	**Materials and methods**	The acute toxicity of the test item DCPP to *Daphnia magna* was determined in a 48-hour static test according to the Commission Directive 92/69/EEC, Annex Part C.2, and the OECD Guideline for Testing of Chemicals No. 202.

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

		The nominal test concentrations were 0.1, 0.22, 0.46, 1.0, and 2.2 mg/L, and a control.	
5.2	**Results and discussion**	In the control and up to and including the test item concentration of nominal 0.46 mg/L no immobility or mortality of the test animals or other signs of intoxication were determined during the test period of 24 hours. After 48 hours of exposure the toxicity of the test item to *Daphnia magna* had increased. The 48h-NOEC was 0.22 mg/L.	
		All reported results are related to total mean measured concentrations of the test item which were in the range of 81-87% of the nominal values.	x
5.2.1	NOEC	0.22 mg a.s./L	
5.2.2	EC_{50}	0.32 mg a.s./L (95% C.I. = 0.22-0.46 mg/L)	
5.2.3	EC_{100}	0.46 mg a.s./L	
5.3	**Conclusion**	The test is considered as valid. The validity criteria are summarised in table A7_4_1_1-8.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	-	x

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2010
	2.1: *Correction*: Replace "(1992)" with "(1984)"
Materials and Methods	3.4.2: Test organisms: in table A7_4_1_2-3: information regarding kind of food, amount of food, feeding frequency and pre-treatment is missing
	3.4.5: Test conditions: in table A7_4_1_2-5: *Correction*: Shift the phrase "light intensity at light period between 200 and 1200 Lux" from section "Photoperiod" to section "Quality/Intensity of Irradiation"
Results and discussion	Agree with applicant's version.
Conclusion	5.2: *Correction*: Rephrase "All reported results are related to total mean measured concentrations of the test item which were in the range of 81-87% of the nominal values." by "All reported biological results are related to the nominal test item concentrations."
	5.3.2: No information given regarding kind of food, amount of food, feeding frequency and pre-treatment (see also 3.4.2)
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Predates recent version Guideline No. 202, adopted on 13 April 2004: Hence, differences occur in respect to the updated version.

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No solvent used
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	Reconstituted test water: analytical grade salts were dissolved in purified water to obtain the following nominal concentrations: $CaCl_2$ x $2H_2O$: 2.0 mmol/ L (= 294 mg/L) $MgSO_4$ x $7H_2O$: 0.5 mmol/ L (= 123 mg/L) $NaHCO_3$: 0.75 mmol/ L (= 65 mg/L) KCl: 0.075 mmol/ L (= 5.8 mg/ L)
Alkalinity ($CaCO_3$)	0.8 mmol/L
Hardness ($CaCO_3$)	2.5 mmol/L (= 250 mg/L) as $CaCO_3$
pH	Measured during the test (see table A7_4_1_1-5)
Ca / Mg ratio	4:1 (based on molarity)
Na / K ratio	10:1 (based on molarity)
Oxygen content	The test water was aerated until oxygen saturation was reached.
Conductance	No data
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna* Straus
Source	Original source: [redacted]
Age (at start of the study)	At the start of the test daphnids were 6-24 hours old and were not first brood progeny
Breeding method	The *Daphnia magna* were cultured in reconstituted water of identical quality (regarding pH, main ions and total hardness) and under identical temperature and light conditions as in the tests.
Kind of food	-
Amount of food	-
Feeding frequency	-
Pre-treatment	-
Feeding of animals during test	Daphnia were not fed during the test

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	The test was performed under static conditions
Volume of test vessels	100 mL glass beakers
Volume/animal	50 mL / 10 animals
Number of animals/vessel	10/vessel
Number of vessels/ concentration	2 replicates per concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20 to 21°C
Dissolved oxygen	> 8.5 mg/L during the test
pH	7.8 to 7.9
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	No data
Photoperiod	16 hours light and 8 hours dark; light intensity at light period between 200 and 1200 Lux

Table A7_4_1_2-6: Influence of DCPP on the mobility of *Daphnia magna*

Nominal concentration of FAT 80'220/A	Sampling date	Age of sample	RCC sample code	FAT 80'220/A measured			
						mean	
[mg/l]	[day]	[hours]		[mg/l]	[% of nominal]	[mg/l]	[% of nominal]
Treatment samples							
0.22	0	0	D-5	0.180	82		
	0	0	D-6	0.179	81	0.18	82
	2	48	D-17	0.178	81		
	2	48	D-18	0.180	82	0.18	81
					total mean :	0.18	81
0.46	0	0	D-7	0.401	87		
	0	0	D-8	0.398	87	0.40	87
	2	48	D-19	0.401	87		
	2	48	D-20	0.398	87	0.40	87
					total mean :	0.40	87
Stock solution sample							
10	0	0	D-25	8.68	87		
Biological control samples							
0	0	0	D-2	n.d.	n.a.	n.a.	n.a.
	2	48	D-14	n.d.	n.a.	n.a.	n.a.
Spiked test water samples							
0.227		0	DZ1	0.235	104		
		0	DZ2	0.235	104	0.235	104
0.454		0	DZ3	0.478	105		
		0	DZ4	0.476	105	0.477	105
					total mean :		104
Analytical blank							
0		0	DZ5	n.d.	n.a.	n.a.	n.a.

Table A7_4_1_2-7: Influence of DCPP on the mobility of *Daphnia magna*

Nominal concentration (mg/l)	No. of daphnids tested	No. of immobilized daphnids after		% of immobilized daphnids after	
		24 h	48 h	24 h	48 h
Control	20	0	0	0	0
0.1	20	0	0	0	0
0.22	20	0	0	0	0
0.46	20	0	20	0	100
1.0	20	20	20	100	100
2.2	20	20	20	100	100

Table A7_4_1_2-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	**Not fulfilled**
Immobilisation of control animals <10%	**X**	
Control animals not staying at the surface	**X**	
Concentration of dissolved oxygen in all test vessels >3 mg/L	**X**	
Concentration of test substance ≥ 80% of initial concentration during test	**X**	

Criteria for poorly soluble test substances	**X**	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](2003). Determination of 48hEC50$_i$ of [redacted] in an Acute Immobilization Test with *Daphnia magna*. [redacted] Report no. [redacted] Date: 2003-03-31 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes - OECD No. 202, Part I (1984)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes - No data are available on immobilisation of daphnids after 24 h which is compulsory according to OECD 202.

3 MATERIALS AND METHODS

3.1	**Test material**	[redacted](Methyldiclosan, degradation product of Diclosan)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	-
3.1.3	Purity	99.7%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-appearance: white powder -water solubility: approx. 0.4 mg/L -homogeneous and stable, not volatile
3.1.6	Method of analysis	Gas chromatography (GC) -solvent: iso-octane -internal standard: hexychlorbenzene -gas chromatograph: GC hp 6890 A, Agilent Technologies -column: Phen_ex 7HG-G002-11, 30 m, 0.25 mm, 0.25 µm -GC method: 02080201G -linear sector of method: 10-50 mg/L -R of calibration function: 0.9997 -standard deviation of injection: ± 3.6%

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

		-recovery rate (water) at 90%: 77±6% -validation report: [redacted]	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A super-saturated stock solution of nominal 100 mg/L was prepared for the nominal test concentration of 100 mg/L. See also table A7_4_2_2-1.	
3.3	**Reference substance**	Yes Potassium dichromate ($K_2Cr_2O_7$, CAS 7778-50-9)	
3.3.1	Method of analysis for reference substance	Not performed	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_1_2-2	x
3.4.2	Test organisms	*Daphnia magna* (see table A7_4_1_2-3)	
3.4.3	Test system	See table A7_4_1_2-4	x
3.4.4	Test conditions	See table A7_4_1_2-5	x, x
3.4.5	Duration of the test	48 h	
3.4.6	Test parameter	Immobilisation	
3.4.7	Sampling	Sampling was performed after 0 and 48 h. No information is given on sample storage.	
3.4.8	Monitoring of TS concentration	Yes – 0 and 48 h	
3.4.9	Statistics	Not applicable since no significant immobilisation occurred.	
		4 RESULTS	
4.1	**Limit Test**	Performed	
4.1.1	Concentration	Loading: 100 mg/L; measured: 0.30 mg/L Control: measured: 0 mg/L	
4.1.2	Number/ percentage of animals showing adverse effects	5%	
4.1.3	Nature of adverse effects	Immobility	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Loading: 100 mg/L; measured: 0.30 mg/L Control: measured: 0 mg/L	
4.2.2	Actual concentrations of	measured:	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

	test substance	- initial (0 h): 0.30 mg/L - final (48 h): 0.007 mg/L; recovery based on initial concentration: 2.4%; - mean measured: 0.15 mg/L - Control: initial (0 h): 0 mg/L: final (48 h): 0.026 mg/L	x
4.2.3	Effect data (Immobilisation)	Test item: 1 animal was immobilised after 48 h (5%). No data are available after 24 h. 48-h NOEC = 0.15 mg/L (mean measured) 48-h EC50 > 0.15 mg/L (mean measured) 48-h EC100 > 0.15 mg/L (mean measured)	x
4.2.4	Concentration / response curve	Not applicable	
4.2.5	Other effects	Not observed	
4.3	**Results of controls**	Control: 0 animals were immobilised after 48 h.	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	7 concentrations between 0.625 and 3.0 mg/L	
4.4.2	Results	24-h EC50 = 1.6 mg/L	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute toxicity of the test item M-DCPP to *Daphnia magna* was determined in a 48-hour static test according to the OECD Guideline for Testing of Chemicals No. 202, part I (1984). The test solution was prepared as a super-saturated solution of 100 mg/L (loading). Prior to use the solution was filtered (0.45 µm). Test concentrations were analytically monitored after 0 and 48 h by gas-chromatography. Limit of detection and/or limit of quantification were not given in the report. Observation on symptoms were made after 48 h only.
5.2	**Results and discussion**	The analytically determined test item concentration was 0.30 mg/L at test start and 0.007 mg/L at test end. The recovery rate based on the initial concentration was 2.4%. Because of the chemical properties of M-DCPP, it was assumed that this was not caused by instability of the test item but by potentially high adsorption and bioaccumulation in the test animals. In the original report the effect values were based on the initial test concentration. Since effects were observed only after 48 h, the effect values were re-calculated to the mean measured concentration (0.15 mg/L). As test item was detected in the control solution at test end, either the control was contaminated or the analytical method was not appropriate to determine exact results. The concentration in the control was even higher than in the corresponding test item solution. No explanation is

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

		given in the report. 1 animal died in the test solution resulting in an immobilisation of 5% after 48 hours. The 48-h EC50 was determined to be > 0.15 mg/L based on mean measured concentrations.	
5.2.1	EC_0	= 0.15 mg/L (mean measured)	x
5.2.2	EC_{50}	> 0.15 mg/L (mean measured)	x
5.2.3	EC_{100}	> 0.15 mg/L (mean measured)	x
5.3	**Conclusion**	The validity criteria can be considered to be fulfilled with the exception of maintaining the test concentration at a satisfactory level (±80% of initial concentration). As the substance has a high adsorption as well as bioaccumulation potential, the test design was not appropriate at keeping the test item level within acceptable limits. See table A7_4_1_2-6.	
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes	x
		- Test concentrations: The test concentrations were not maintained satisfactorily. The recovery is very low: 2.4% of initial concentration (0 h: 0.30 mg/L; 48 h: 0.007 mg/L).	
		- In addition, the concentration at test end is even lower than the concentration in the control. No explanation is given.	x
		- No analytical limits (LOD/LOQ) are given in the report.	
		- Calculation of effective concentrations: The effective concentrations are based on the initially measured concentrations. Since the fate of the test item is not proven and no effects were observed during the test, the effect values should be based on average concentrations. This is a methodological error.	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA7.2 *Daphnia magna*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2013
Materials and Methods	3.4.1 in table A7_4_1_2-2: *Addition to "pH" box:* "After pH adjustment for use as dilution water: 7.8 ± 0.2
	3.4.3 in table A7_4_1_2-4 in box "Breeding method": Delete "feeding with"
	3.4.4 in table A7_4_1_2-5 in "Dissolved oxygen" box: Exchange "8.9 to 9.1" with "8.6 to 8.7" and exchange "8.4 to 8.6" with "8.6 to 8.8".
	3.4.4 in table A7_4_1_2-5 in "pH" box: Delete "to 7.9" and exchange "7.8 to 8.1" with "7.7 to 7.9".
Results and discussion	4.2.2: Replace "0.15 mg/L" by "0.046 mg/L based on geometric mean from measured concentrations at test beginning and end".
	4.2.3: Replace all concentrations given with "0.15" by "0.046".
Conclusion	5.2.1: Replace "0.15 mg/L (mean measured)" by "0.046 mg/L based on geometric mean from measured concentrations at test beginning and end".
	5.2.2: Replace "0.15 mg/L (mean measured)" by "0.046 mg/L based on geometric mean from measured concentrations at test beginning and end".
	5.2.3: Replace "0.15 mg/L (mean measured)" by "0.046 mg/L based on geometric mean from measured concentrations at test beginning and end".
	5.3.2: *Addition*: "Some parameters like alkalinity or hardness are not provided in the test report."
	5.3.2: Replace "No explanation is given." by "The occurrence of Methyl-DCPP in the control was due to an error in the analytical measurement according to the study authors."
Reliability	2
Acceptability	Acceptable for environmental risk assessment with amendments given above
Remarks	Although the study has several deficiencies including the decline of the test substance a use seems plausible when applying the geometric mean of the measured concentrations.

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No solvent used
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	Filtration (0.45 µm)

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	M4-medium (Drinking water with an enrichment of minerals is used in the test (calcium chloride, magnesium sulphate, sodium chloride, potassium chloride).)
Alkalinity	No data
Hardness	Not specified
pH	Drinking water: 8.28
Ca / Mg ratio	Not specified
Na / K ratio	Not specified
Oxygen content	No data
Conductance	246 µS/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna (STRAUS)*
Source	[redacted]
Age	0 to 24 h
Breeding method	Females multiplied by parthenogenesis Holding: glass beakers (2 L), medium M4 (Elendt), feeding with, change of medium twice a week, photo period 16/8 h, T = 20±1°C
Kind of food	Tetra Aminin® suspended in M4 and green algae *Desmodesmus subspicatus*
Amount of food	No data
Feeding frequency	No data
Pretreatment	30-min settling-in period (animals which showed no apparent damage were used in the test)
Feeding of animals during test	No

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Not applicable
Volume of test vessels	Glass beakers, nominal volume 50 mL, tall shape
Volume/animal	4 mL/animal
Number of animals/vessel	5
Number of vessels/ concentration	4
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_2-5: Test conditions

Criteria	Details
Test temperature	20±1 °C
Dissolved oxygen	Test solutions: 0 h: 8.9 to 9.1 mg/L; 48 h: 8.4 to 8.6 mg/L
pH	Test solutions: 0 h: 7.8 to 7.9; 48 h: 7.8 to 8.1
Adjustment of pH	No
Aeration of dilution water	No
Quality/Intensity of irradiation	No lighting used
Photoperiod	e.g. 12 h photoperiod daily

Table A7_4_1_2-6: Validity criteria for acute daphnia immobilistaion test according to OECD Guideline 202

	fulfilled	Not fullfilled
Immobilisation of control animals <10%	X	
Control animals not staying at the surface	X	
Concentration of dissolved oxygen in all test vessels >3 mg/l	X	
Concentration of test substance ≥80% of initial concentration during test		X

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1999d): Acute toxicity of [redacted] to *Scenedesmus subspicatus* in a 72-hour algal growth inhibition test. [redacted], Report No. [redacted] date: 1999-04-06 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Directive 92/69/EEC, C.3 and OECD No. 201 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](DCPP)	
3.1.1	Lot/Batch number	Batch No. [redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	>99%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Stability in water: >1 year (estimated half-life at 25 °C; determined in RCC Project 712260) Solubility in water: 19.5 mg/L (determined in RCC Project 712012)	
3.1.6	Method of analysis	HPLC-UV/VIS	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A stock solution of nominal 10 mg/L was prepared by dissolving 20 mg of test item in 2000 mL test water by ultrasonic treatment (15 minutes) and intense stirring by a magnetic stirrer (72 hours at room temperature in the dark). The stirring period was chosen to make sure that the test item was completely dissolved and was based on the results of pre-tests (without GLP) where a solubility of about 10-20 mg/L and a stability of at least 96 hours were determined. Adequate volumes of this intensively mixed stock solution were added to test media of the following nominal test concentrations: 2.2, 4.6, 10, 22, and 46 mg/L. See also table A7_4_1_3-1.	x x
3.3	**Reference substance**	No	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Culture medium	The algae were cultivated and tested in synthetic test water, prepared according to the mentioned test guidelines: Analytical grade salts were added to sterile purified water to obtain the following final nominal concentrations: <u>Macronutrients stock solution</u> $NaHCO_3$ 50.0 mg/L $CaCl_2$ x $2H_2O$ 18.0 mg/L Na_4Cl 15.0 mg/L $MgSO_4$ x $7H_2O$ 15.0 mg/L $MgCl_2$ x $6H_2O$ 12.0 mg/L KH_2PO_4 1.6 mg/L <u>Trace elements</u> Na_2EDTA x$2H_2O$ 100.0 µg/L $FeCl_3$ x$6H_2O$ 80.0 µg/L $MnCl_2$ x$4H_2O$ 415.0 µg/L H_3BO_3 185.0 µg/L Na_2MoO_4 x$2H_2O$ 7.0 µg/L $ZnCl_2$ 3.0 µg/L $CoCl_2$ x$6H_2O$ 1.5 µg/L $CuCl_2$ x$2H_2O$ 0.01 µg/L Water hardness: 0.24 mmol/L (=24 mg/L) as $CaCO_3$	
3.4.2	Test organisms	See table A7_4_1_3-1	
3.4.3	Test system	See table A7_4_1_3-2	
3.4.4	Test conditions	See table A7_4_1_3-3	
3.4.5	Duration of the test	72 hours	
3.4.6	Test parameter	The test substance concentration at which there was 50 % inhibition of growth of biomass (E_bC_{50}). The 50% inhibition of the growth rate (E_rC_{50}) was not measured. Also detected were the lowest concentration at which there was an observable effect (LOEC) and the concentration at which there was no observed effect (NOEC).	x
3.4.7	Sampling	Small volumes of the test media and the control (1.0-2.0 mL) were taken out of all test flasks after 24, 48, and 72 hours of exposure and were not replaced. The algae cell densities in the samples were determined by counting with an electronic particle counter with at least two measurements per sample. In addition, after the test period of 72 hours, a sample was taken from the control and from the test concentration of nominal 10 µg/L. The shape of the algal cells was microscopically examined. The pH values were measured in samples from all test concentration and the control at the start and at the end of the test. The water temperature	

		was measured at least daily in an Erlenmeyer flask filled with water and incubated under the same conditions as the test flasks. The appearance of the test media was recorded daily.	
3.4.8	Monitoring of TS concentration	For the analytical measurements of the test item concentrations, one sample from the freshly prepared stock solution and duplicate samples from the freshly prepared test media of all test concentrations and from the control were taken just before the start of the test (without algae). For the determination of the stability of the test item under the test conditions, and for the maintenance of the test item concentrations during the test period respectively, additional flasks with adequate volumes of the freshly prepared test media of all test concentrations and the control were incubated under the same conditions as in the actual test (but without algae). And were sampled in duplicate at the end of the test (after the 72 hours test period). All samples were deep-frozen (at about -20 °C) immediately after sampling. The concentrations of the test item DCPP were analyzed in the stock solution sample and in the duplicate test media samples from the test concentrations of nominal 10-46 µg/L from both sampling times (0 and 72 hours). From the control samples only one of the duplicate samples was analysed from each of both sampling times (0 and 72 hours). The samples from the test concentrations were below the determined 72-hour NOEC.	
3.4.9	Statistics	The E_bC_{50} and E_rC_{50} (the concentration of the test item corresponding to 50% inhibition of algal biomass b respectively growth rate µ compared to the control), and the corresponding EC_{10} and EC_{90} and their 95%-confidence limits were calculated by probit Analysis. For the determination of the LOEC and NOEC, the calculated mean biomass and the mean growth rate µ at the test concentrations were tested on significant differences to the control values by a Dunnett-test.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	The test concentrations were based on the results of a range-finding test and the results of a pre-experiment to the solubility of the test item (without GLP). Nominal concentrations: 2.2, 4.6, 10, 22, and 46 mg/L and control.	x

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

4.2.2	Actual concentrations of test substance	In the sample from the freshly prepared stock solution 104% of the nominal concentration was measured. In the test media the mean measured test item concentrations at the start of the test ranged from 112 to 124% of the nominal values. During the test period of 72 hours a slight decrease of the test item concentration in the test media was determined. At the end of the test 77 to 114% of the nominal values were found. The losses could be due to an adsorption of the test item onto glass surfaces, particularly in the low-level samples. However, the total mean measured test concentrations (calculated as the average over all measurements per test concentration) varied in the range of 95 to 119% of the nominal values. Therefore, all biological results are related to the nominal concentrations of the test item. See table A7_4_1_3-4	x x x
4.2.3	Growth curves	Growth curves (number of cells vs. time) are given in the report on page 33.	
4.2.4	Concentration / response curve	Not given in the report	
4.2.5	Cell concentration data	See table A7_4_1_3-5	
4.2.6	Effect data (cell multiplication inhibition)	See table A7_4_1_3-6	
4.2.7	Other observed effects	The shape of the algal cells growing up to 10 µg/L was obviously not affected.	x
4.3	**Results of controls**	See table A7_4_1_3-5	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	--	
4.4.2	Results	--	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of the test item DCPP on the growth of the green algal species *Scenedesmus subspicatus* CHODAT was investigated in a 72-hour static test according to the Commission Directive 92/69/EEC, Annex Part C.3, and the OECD Guideline for Testing of Chemicals No. 201. The nominal test concentrations were 2.2, 4.6, 10, 22, and 46 µg/L, and in parallel a control.	
5.2	**Results and discussion**	The analytically determined test item concentration in the analysed test media ranged from 77 to 120% of the nominal values. The total mean measured test item concentrations were in the range of 95 to 119% of the nominal values. Therefore, the reported biological results are based on the nominal concentrations of the test item.	x x x
5.2.1	NOEC	10 µg/L	x

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Desmodesmus subspicatus* (former name*: Scenedesmus subspicatus*)

5.2.2	EC_{50}	E_bC_{50} = 23 µg/L E_rC_{50} = 38 µg/L	x
5.3	**Conclusion**	A clear dose – response relationship can be derived from the cell concentration data. Validity criteria are summarised in table A7_4_1_3-8.	x
5.3.1	Reliability	1	x
5.3.2	Deficiencies	-	x

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	November 2010
Materials and Methods	3.2: *Correction*: µg instead of mg.
	3.2: *Deletion*: Delete phrase "See also table A7_4_1_3-1."
	3.4.6: *Replacement*: Replace "The test substance concentration at which there was 50 % inhibition of growth of biomass (E_bC_{50}). The 50% inhibition of the growth rate (E_rC_{50}) was not measured." by "The test substance concentrations at which there was 50 % inhibition of growth of biomass (E_bC_{50}) and 50% inhibition of the growth rate (E_rC_{50}) were calculated."
Results and discussion	4.2.1: C*orrection*: µg instead of mg.
	4.2.2: Correction: Delete term "slight" in sentence "During the test period of 72 hours a slight decrease of the test item concentration in the test media was determined".
	4.2.2: *Replacement*: Replace phrase "However, the total mean measured test concentrations (calculated as the average over all measurements per test concentration) varied in the range of 95 to 119% of the nominal values. Therefore, all biological results are related to the nominal concentrations of the test item" by "As 80% of the initial test item concentration could not be maintained over all test concentrations trough out test duration the geometric mean was used for determination of the NOEC. For determination of E_rC_{50})and E_bC_{50} nominal concentrations were used (the total mean measured test concentrations, calculated as the average over all measurements per test concentration, varied in the range of 95 to 119.5% of the nominal values)."
	4.2.2: *Correction*: 22 µg/L nominal concentration instead of 21 µg/L in table A7_4_1_3-4
	4.2.7: *Insertion*: Insert "based on nominal concentration following term "10 µg/L".

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA VII.7.3 *Scenedesmus Subspicatus*

Conclusion	5.2: *Correction*: Replace "120%" by "124%" 5.2: *Replacement*: Replace phrase "Therefore, the reported biological results are based on the nominal concentrations of the test item." by "As 80% of the initial test item concentration could not be maintained over all test concentrations trough out test duration the geometric mean was used for determination of the NOEC" 5.2.1: *Correction*: Replace "10 µg/L" by 9.3 µg/L" based on geometric mean of measured concentrations". 5.2.2: *Addition*: "values based on calculations using nominal concentrations". 5.3: *Correction*: Replace table name from A7_4_1_3-8 to A7_4_1_3-7 and replace table name in Table A7_4_1_3-6 Validity criteria for algal growth inhibition test according to OECD Guideline 201 by "A7_4_1_3-7 Validity criteria for algal growth inhibition test according to OECD Guideline 201" Table 7_4_1_3-7: *Correction*: Change cross for the criteria "Concentration of test substance ≥ 80% of initial concentration during test" from "Fulfilled" to "Not Fulfilled". 5.3.1: Change to "2" 5.3.2: *Addition:* Test item concentration was not maintained within 80% of the test item concentration. Graphical presentation of the concentration effect relationship missing.
Reliability	2 as the study is a GLP study conducted according to internationally accepted guidelines but the test item concentrations could not be maintained within 80% of the initial test item concentrations through out test duration. Nevertheless, a reliable NOEC of 9.3 µg/L derived as a geometric mean based on measured concentrations at the beginning and end of the test could be obtained, which did not differ in a very considerable amount from the nominal test concentration of 10 µg/L.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Predates recent version of Guideline No. 201, adopted on 23 March 2006: Hence, some differences occur in respect to the updated version: e.g 80.0 µg/L FeCl3 x6H2O were used for the media and the test started with a biomass of 1 x 10^4 cells per mL of test solution.

Table A7_4_1_3-1: Test organisms

Criteria	Details
Species	*Desmodesmus subspicatus* (former name: *Scenedesmus subspicatus)*
Strain	[redacted]
Source	Supplied by the '[redacted]
Laboratory culture	Yes
Method of cultivation	The algae had been grown in the[redacted] under standardised conditions according to the test guidelines.
Pre-treatment	The algal culture used for the toxicity test was 3 days old and had been maintained under the same conditions as those for the toxicity test.
Initial cell concentration	Test started with a biomass of. 10,000 (= 1 x 10^4) cells per mL of test solution.

Table A7_4_1_3-2: Test system

Criteria	Details
Volume of culture flasks	50 mL Erlenmeyer flasks with 15 mL algal suspension covere d with glass dishes
Culturing apparatus	Incubation was performed under standardised conditions according to the mentioned guidelines
Light quality	Continuous illumination (illumination by fluorescence tubes in a distance of about 35 cm from the test flask)
Procedure for suspending algae	Constant stirring by magnetic stirrers
Number of vessels/ concentration	Three replicates per test concentration and six replicates in the control.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-3: Test conditions

Criteria	Details
Test temperature	23°C
pH	Ranged from 7.8 to 8.8
Aeration of dilution water	No
Light intensity	8147 Lux (mean value, range between: 7820 and 8620 Lux)
Photoperiod	Continuous illumination in the incubator (24 h/day)

Table A7_4_1_3-4: Results obtained for the concentration of the test item in the test medium

Nominal concentration of FAT 80'220/A	Sampling date	Age of sample	RCC sample code	FAT 80'220/A measured			
						mean	
[µg/l]	[day]	[hours]		[µg/l]	[% of nominal]	[µg/l]	[% of nominal]
Treatment samples							
10	0	0	A7	11.2	112		
	0	0	A8	11.2	112	11.2	112
	3	72	A19	7.46	74.6		
	3	72	A20	7.99	79.9	7.72	77
					total mean :	9.47	95
21	0	0	A9	25.5	121		
	0	0	A10	26.8	128	26.1	124
	3	72	A21	23.4	112		
	3	72	A22	24.7	117	24.0	114
					total mean :	25.1	119
46	0	0	A11	52.7	115		
	0	0	A12	50.9	111	51.8	113
	3	72	A23	38.7	84.1		
	3	72	A24	38.2	83.1	38.4	84
					total mean :	45.1	98
Stock solution sample							
10000	0	0	A25	10352	104		
Biological control samples							
0	0	0	A1	n.d.	n.a.	n.a.	n.a.
	3	72	A13	n.d.	n.a.	n.a.	n.a.
Spiked test water samples							
9.46		0	AZ1	9.26	97.9		
71.0		0	AZ2	72.5	102		
					total mean :		100
Analytical blank							
0		0	AZ3	n.d.	n.a.	n.a.	n.a.

Table A7_4_1_3-5: Algal cell densities during the test period of 72 hours

Nominal conc. (µg/l)	Flask No.	Density of algal cells (cell number x 10,000/ml) after 24 h		48 h		72 h	
control	1	7.0	7.0	25.8	25.7	103.1	101.2
	2	6.6	6.2	22.9	22.8	88.1	88.4
	3	6.6	6.8	24.6	24.8	93.9	94.3
	4	7.7	7.5	23.7	23.5	98.4	99.1
	5	6.5	6.5	22.0	21.4	96.4	97.2
	6	6.2	6.0	22.1	22.1	93.7	93.1
	m	6.7		23.5		95.6	
	s	0.5		1.6		4.8	
	n	6		6		6	
2.2	1	6.8	6.3	26.7	26.9	110.3	109.1
	2	6.6	6.8	27.5	27.7	114.0	115.0
	3	6.7	6.2	24.7	25.2	113.8	113.6
	m	6.6		26.5		112.6	
	s	0.1		1.4		2.6	
	n	3		3		3	
4.6	1	6.3	6.5	27.3	27.4	107.2	106.3
	2	6.5	6.3	24.0	23.9	96.8	95.9
	3	5.8	6.0	25.0	24.6	119.7	118.7
	m	6.2		25.4		107.4	
	s	0.3		1.8		11.4	
	n	3		3		3	
10	1	8.0	8.0	27.3	26.6	98.1	98.0
	2	7.0	7.0	24.8	24.2	108.8	107.9
	3	5.7	5.3	20.0	19.5	81.0	80.6
	m	6.8		23.7		95.7	
	s	1.3		3.7		13.9	
	n	3		3		3	
22	1	3.4	3.5	12.2	12.3	43.4	42.1
	2	4.0	3.9	10.2	10.3	54.6	54.0
	3	3.6	3.3	11.2	11.4	49.9	50.8
	m	3.6		11.3		49.1	
	s	0.3		1.0		5.9	
	n	3		3		3	
46	1	3.8	3.4	2.9	2.9	6.1	5.7
	2	2.5	2.6	2.4	2.2	6.8	6.3
	3	2.6	2.6	2.7	2.5	3.6	3.5
	m	2.9		2.6		5.3	
	s	0.6		0.3		1.6	
	n	3		3		3	

Table A7_4_1_3-6: Results of DCPP to *Desmodesmus subspicatus* based on nominal concentrations

Parameter (0-72 h)	Biomass b (µg/l)	Growth rate µ (µg/l)
EC50	23	38
EC10	14	20
EC90	40	73*

Table A7_4_1_3-6: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X	
Concentration of test substance ≥ 80% of initial concentration during test	X	

Criteria for poorly soluble test substances	X	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted]. Determination of 72h EC50 of [redacted] using *Desmodesmus subspicatus*. [redacted] Report no. [redacted] Date: 2003-07-30 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Not specified, presumably OECD No. 201	x
2.2	**GLP**	Yes	
2.3	**Deviations**	No guideline specified	x
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](Methyldiclosan, degradation product of Diclosan)	
3.1.1	Lot/Batch number	No data	x
3.1.2	Specification	Deviating from specification given in section 2 as follows	
3.1.3	Purity	No data	x
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	No data	
3.1.6	Method of analysis	Gas chromatography (GC)	
		- solvent: iso-octane	
		- internal standard: hexachlorbenzene	x
		- gas chromatograph: GC hp 6890 A, Agilent Technologies	
		- column: Phen_ex 7HG-G002-11, 30 m, 0.25 mm, 0.25 µm	
		- GC method: 02080201G	
		- linear sector of method: 10-50 mg/L	x
		- R of calibration function: 0.9997	
		- standard deviation of injection: ± 3.6%	
		- recovery rate (water) at 0.2 mg/L: 96±3%	
		- recovery rate (water) at 0.4 mg/L: 97±3%	
		- validation report: VB02080201G926	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A super-saturated stock solution of the test item was prepared. This was done by mixing 100 mg/L of the test item with dilution water and shaking vigorously for 24 hours. The resulting solution was filtrated through 0.45 µm filters. The stock solution then was used to prepare the solutions of the concentrations to be tested. See also table A7_4_1_3-1.
3.3	**Reference substance**	No
3.3.1	Method of analysis for reference substance	Not applicable
3.4	**Testing procedure**	
3.4.1	Culture medium	Not specified
3.4.2	Test organisms	*Desmodesmus subspicatus* (see table A7_4_1_3-2)
3.4.3	Test system	See table A7_4_1_3-3
3.4.4	Test conditions	See table A7_4_1_3-4
3.4.5	Duration of the test	72 h
3.4.6	Test parameter	Cell concentration (determined by photometric measurement at 440 nm)
3.4.7	Sampling	Every 24 h
3.4.8	Monitoring of TS concentration	Yes (after 0 and 72 h)
3.4.9	Statistics	Calculation of area under growth curve and specific growth rate
		4 RESULTS
4.1	**Limit Test**	Not performed
4.1.1	Concentration	Not applicable
4.1.2	Number/ percentage of animals showing adverse effects	Not applicable
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	Nominal:12, 17.6, 25.6, 36.8, 54.4, 80% saturation
4.2.2	Actual concentrations of test substance	

% saturation	**Measured test item concentrations (mg/L)**			**recovery after 72 h (%initial)**
	0 h	**72 h**	**mean**	
0 (control)	0	0.006	-	-
12	0.022	0.003	0.013	14%
17.6	0.039	0.004	0.022	10%

x

Section A7.4.1.3 Growth inhibition test on algae

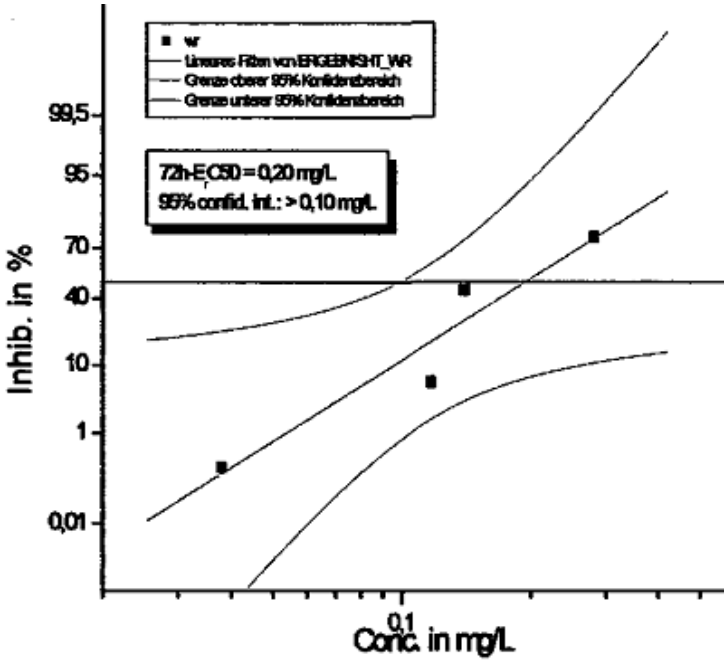
Annex Point IIA7.3

25.6	0.032	0.005	0.019	16%	
36.8	0.12	0.008	0.064	7%	
54.4	0.14	0.003	0.072	2%	
80	0.28	0.11	0.195	39%	

4.2.3 Growth curves — Not available

4.2.4 Concentration / response curve — See table A7_4_1_3-6 — x

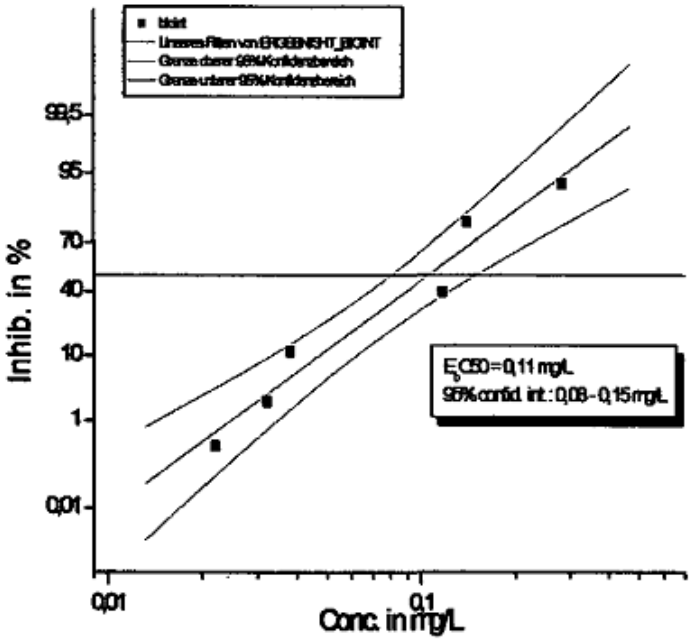
Growth rate:



Area under curve:

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3



4.2.5	Cell concentration data	See table A7_4_1_3-5	
4.2.6	Effect data (cell multiplication inhibition)		x
4.2.7	Other observed effects	No data	
4.3	**Results of controls**	See table A7_4_1_3-5 and table A7_4_1_3-6	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	

	Initial test concentration	**Mean measured test concentration**
72h NOEC	0.022 mg/L	0.013mg/L
72h ErC50	0.20 mg/L (95% CI > 0.10)	approx. 0.072 mg/L
72h EbC50	0.11 mg/L (95% CI 0.08 – 0.15)	approx. 0.064 mg/L

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The acute toxicity of the test item M-DCPP to *Desmodesmus subspicatus* was determined in a 72-hour static test according to the OECD Guideline for Testing of Chemicals No. 201.	x
		The nominal test concentrations were 12, 17.6, 25.6, 36.8, 54.4 and 80	

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

		%saturation based on a super-saturated stock solution of 100 mg/L and a control. The test solutions were made by diluting the stock solution after filtration (0.45 µm). The test concentrations were analytically determined after 0 and 72 h via GC. Cell concentration was determined via absorption at 440 nm every 24 h. Specific growth rate and area und curve were evaluated for the inhibition of the algal growth by M-DCPP.	
5.2	**Results and discussion**	A significant inhibition of the specific growth rate was found in the two highest test concentrations (54.4 and 80% saturation; 0.072 and 0.195 mg/L, mean measured). Concerning the area under the growth curve (AUC), the inhibition considerably starts from 36.8 mg/L (nominal), 0.12 mg/L (measured) respectively. According to the authors of the study, the AUC is assumed to be the more sensitive endpoint. All reported results are related to total mean measured concentration of the test item at begin of the test.	x x
5.2.1	NOE_rC	0.13 mg/L (mean measured)	x
5.2.2	E_{r50}	approx. 0.072 mg/L (mean measured)	x
5.2.3	E_bC_{50}	approx. 0.064 mg/L (mean measured)	x
5.3	**Conclusion**	The test is considered as valid since the replication factor in the blank control is 26 (demanded at least 16 in 72h) and the pH in the blank control changed by 0.6 units (demanded: not higher than 1.5 units in 72h). The test concentrations were not satisfactorily maintained during the exposure phase (±80% of initial concentration). As the substance has a high adsorption as well as bioaccumulation potential, the test design was not appropriate at keeping the test item level within acceptable limits.	
5.3.1	Reliability	3	x
5.3.2	Deficiencies	Yes - As this is just an amendment to a previously conducted study and not the complete report study details are missing. - Test concentrations: The test concentrations were not maintained satisfactorily. The recovery is low (2% to 39%; mean 15%). - No analytical limits (LOD/LOQ) are given in the report. - Calculation of effective concentrations: The effective concentrations are based on the initially measured concentrations. Since the fate of the test item is not proven and no effects were observed during the test, the effect values should be based on average concentrations. This is a methodological error.	x

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2013
Materials and Methods	Ad 2.1*Comment*:test conditions (as far as stated) and validity criteria (e.g. pH stability during the test, minimum requirement of cell concentration increase in controls) are according to OECD No. 201 *Clarification from the final report:* the study was conducted in accordance with the following guidelines: OECD No 201 (1984) and EN 28692. Ad 2.3 *Comment*: As the provided study is an amendment, the guideline could be cited in the final report- clarification is needed. *Clarification from the final report*: the study was conducted in accordance with the following guidelines: OECD No 201 (1984) and EN 28692. Ad 3.1.1 *Comment*: As the provided study is an amendment, Lot/Batch number could be stated in the final report- clarification is needed. *Clarification from the final report*: Batch No.: [redacted] Ad 3.1.3 *Comment*: see Comment to 3.1.1. *Clarification from the final report*: Purity: 99.7%. Ad 3.1.6 *Comment*: the internal standard is not mentioned in the study, as the provided study is an amendment, the internal standard could be stated in the final report- clarification is needed. *Clarification from the final report*: internal standard was neither mentioned in the final report, only the reference of the validation report is provided [redacted] Ad 3.1.6 *Comment*: LOQ and LOD should be clarified- see comment above. *Clarification from the final report*: LOQ and LOD were neither mentioned in the final report, only the reference of the validation report is provided [redacted]
Results and discussion	Ad 4.2.2 *Correction*: for the calculation of the mean measured concentration, the geometric mean should be used. Calculations from the RMS: for 12, 17.6, 25.6, 36.8, 54.4 and 80% saturation the mean measured concentrations are 0.008, 0.012, 0.013, 0.031, 0.020 and 0.18 mg/L, respectively. Ad 4.2.4 *Comment*: Concentration response curves were based on initial measured concentrations and should be corrected to mean measured concentrations. Ad 4.2.6 *Correction*: Based on mean measured concentrations (calculated with the geometric mean), the72h NOEC is estimated for growth rate as 0.013 mg/L and the72h E_rC_{50} as 0.020< 72h E_rC_{50} <0.18mg/L. Furthermore, the 72h NOE_bC is estimated as 0.008 mg/L and the72h E_bC_{50} as 0.020< 72h E_bC_{50} <0.18mg/L

Section A7.4.1.3 Growth inhibition test on algae

Annex Point IIA7.3

Conclusion	Ad 5.1 *Comment*: The OECD No 201 is not mentioned in the provided study. *Clarification from the final report:* the study was conducted in accordance with the following guidelines: OECD No 201 (1984) and EN 28692. Ad 5.2 *Correction*: for 54.4 and 80% saturation the mean measured concentrations (based on geometric mean) are 0.020 and 0.18 mg/L, respectively. Ad 5.1 *Correction*: 36.8% saturation is not according to 36.8 mg/L(nominal). Given a water solubility of 0.322 mg/L (Doc II-A, 1.3.2) the nominal concentration according to 36.8% saturation could be estimated as 0.118 mg/L. Ad 5.2.1 *Correction*: the72h NOErC should be estimated as 0.013 mg/L. Ad 5.2.2 *Correction*: the72h ErC_{50} should be estimated as 0.020< 72h E_rC_{50} <0.18mg/L. Ad 5.2.3 *Comment*: the 72h E_bC_{50} should be estimated as 0.020< 72h E_bC_{50} <0.18mg/L. Ad 5.3.2 *Comment*: Please provide the complete study report to be able to conclude on the deficiencies. *Clarification from the final report*: Concerning the deficiencies there was no additional information from the final report.
Reliability	2 (see comments stated under “Acceptability”)
Acceptability	From the provided information it is assumed, that this study was conducted according to the OECD guidance No 201. Although there were some deficiencies in the study report, the results could be used for an estimation of the toxicity of Methyl-DCPP to algae. Instead of the initial concentrations the mean measured concentrations should be used for the calculations of the effect-concentrations. As the present study is an amendment the final report is required from the applicant to conclude on the acceptability of this study (see points for clarification). *Clarification from the final report*: the study was conducted in accordance with the following guidelines: OECD No 201 (1984) and EN 28692. Purity and Batch No. of the test item could be clarified. Concerning the deficiencies there was no additional information from the final report. Nevertheless the results from this study could be used for an estimation of the toxicity of Methyl-DCPP to algae.
Remarks	Ad Table A7_4_1_3-4 Comment: There were no pH-values stated in the provided study. Only the information was provided that the pH in the control changed 0.6 pH units.

Table A7_4_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Filtration of stock solution prior to use (0.45 µm)

Table A7_4_1_3-2: Test organisms

Criteria	Details
Species	*Desmodesmus subspicatus*
Strain	No data
Source	No data
Laboratory culture	Yes
Method of cultivation	No data
Pretreatment	Four days before the start of the test, an aliquot of the alga stock culture containing a few cells was brought into50 mL pre-culture medium and incubated for 96 hours. The resulting culture was growing exponentially. After adjustment to a cell concentration of about 10^4-$10^{5/}$mL by photometric measurement and addition of pre-culture medium, the culture was usable for the test.
Initial cell concentration	7.61E4 cells/mL

Table A7_4_1_3-3: Test system

Criteria	Details
Culture flasks	Screw cap cuvettes d = 6mm, no data in volume
Culturing apparatus	No data
Light quality	No data
Procedure for suspending algae	No data
Number of vessels/ concentration	6/control; 8/test concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_3-4: Test conditions

Criteria	Details
Test temperature	21±2 °C
pH	7.7 to 7.9
Adjustment of pH	No
Aeration of dilution water	No
Intensity of irradiation	> 5000 lux
Photoperiod	Continuous light

Table A7_4_1_3-5: Cell concentration data (cell data in cells/mL)

% saturation	cell concentration			
	0 h	24 h	48 h	72 h
0	7,61E4	3,67E5	1,50E6	1,97E6
0	7,61E4	3,72E5	1,52E6	2,02E6
0	7,61E4	3,77E5	1,50E6	1,95E6
0	7,61E4	3,82E5	1,58E6	2,02E6
0	7,61E4	3,77E5	1,58E6	2,01E6
0	7,61E4	3,57E5	1,52E6	1,99E6
80	7,61E4	1,51E5	1,71E5	1,96E5
80	7,61E4	1,36E5	1,51E5	1,61E5
80	7,61E4	1,41E5	1,66E5	1,91E5
80	7,61E4	1,31E5	1,76E5	1,66E5
80	7,61E4	1,41E5	1,58E5	1,58E5
80	7,61E4	1,26E5	1,41E5	1,41E5
80	7,61E4	1,36E5	1,36E5	1,46E5
80	7,61E4	1,36E5	1,51E5	1,86E5
80	7,61E4	1,61E5	1,31E5	1,71E5
54,4	7,61E4	1,41E5	1,46E5	4,42E5
54,4	7,61E4	1,86E5	3,37E5	5,78E5
54,4	7,61E4	2,11E5	3,37E5	4,87E5
54,4	7,61E4	1,81E5	2,57E5	3,47E5
54,4	7,61E4	2,01E5	3,22E5	5,52E5
54,4	7,61E4	2,06E5	3,62E5	5,88E5
54,4	7,61E4	1,86E5	2,62E5	3,57E5
54,4	7,61E4	2,21E5	2,87E5	3,92E5
54,4	7,61E4	1,86E5	3,07E5	4,07E5
36,8	7,61E4	3,12E5	7,23E5	1,59E6
36,8	7,61E4	2,82E5	6,68E5	1,49E6
36,8	7,61E4	2,72E5	6,83E5	1,56E6
36,8	7,61E4	2,87E5	7,98E5	1,78E6
36,8	7,61E4	2,77E5	7,08E5	1,70E6
36,8	7,61E4	2,92E5	7,53E5	1,68E6
36,8	7,61E4	2,82E5	6,83E5	1,62E6
36,8	7,61E4	2,87E5	6,63E5	1,52E6
36,8	7,61E4	2,72E5	7,63E5	1,72E6

% saturation	cell concentration			
	0 h	24 h	48 h	72 h
25,6	7,61E4	3,32E5	1,45E6	1,95E6
25,6	7,61E4	3,47E5	1,14E6	1,95E6
25,6	7,61E4	3,37E5	1,23E6	1,97E6
25,6	7,61E4	3,57E5	1,33E6	1,98E6
25,6	7,61E4	3,37E5	1,27E6	1,99E6
25,6	7,61E4	3,42E5	1,08E6	1,94E6
25,6	7,61E4	3,42E5	1,31E6	2,04E6
25,6	7,61E4	3,42E5	1,29E6	2,04E6
25,6	7,61E4	3,47E5	1,28E6	1,94E6
17,6	7,61E4	3,57E5	1,40E6	2,01E6
17,6	7,61E4	3,72E5	1,50E6	2,17E6
17,6	7,61E4	3,67E5	1,45E6	2,02E6
17,6	7,61E4	3,72E5	1,43E6	2,08E6
17,6	7,61E4	3,47E5	1,42E6	2,07E6
17,6	7,61E4	3,32E5	1,38E6	2,04E6
17,6	7,61E4	3,72E5	1,48E6	2,05E6
17,6	7,61E4	3,67E5	1,45E6	2,13E6
17,6	7,61E4	3,67E5	1,47E6	2,08E6
12	7,61E4	3,92E5	1,54E6	2,01E6
12	7,61E4	3,52E5	1,49E6	2,00E6
12	7,61E4	3,87E5	1,56E6	2,03E6
12	7,61E4	3,87E5	1,52E6	1,96E6
12	7,61E4	3,82E5	1,53E6	1,99E6
12	7,61E4	3,77E5	1,58E6	2,07E6
12	7,61E4	3,57E5	1,45E6	1,98E6
12	7,61E4	3,82E5	1,54E6	1,99E6
12	7,61E4	3,47E5	1,48E6	1,92E6

Table A7_4_1_3-6: Area under curve, growth rate and mean values of inhibition

% saturation	Area under the curve		growth rate		mean values of inhibition in %	
	value	inhibition (%)	value	inhibition (%)	AUC	growth rate
0	2,66E6	1,85	1,08	0,39	0	0
	2,71E6	0,19	1,09	0,39		
	2,66E6	1,58	1,08	0,82		
	2,76E6	-2,04	1,09	-0,39		
	2,77E6	-2,32	1,09	-0,31		
	2,69E6	1,11	1,09	0,07		
80	2,31E5	91,47	0,32	70,94	93,1	75,8
	1,78E5	93,42	0,25	76,97		
	2,13E5	92,12	0,31	71,74		
	2,01E5	92,59	0,26	76,04		
	1,98E5	93,14	0,24	77,94		
	1,18E5	94,53	0,21	81,04		
	1,56E6	94,26	0,22	79,97		
	1,91E5	92,96	0,30	72,55		
	1,88E5	93,06	0,27	75,13		
54,4	3,18E5	88,23	0,59	46,09	80,7	45,4
	6,22E5	77,02	0,68	37,91		
	6,02E5	77,76	0,62	43,12		
	4,21E5	84,43	0,51	53,53		
	6,09E5	77,48	0,66	39,27		
	6,72E5	75,16	0,68	37,38		
	4,38E5	83,87	0,52	52,65		
	5,14E5	81,00	0,55	49,78		
	5,07E5	81,28	0,56	48,63		
36,8	1,54E6	99,39	1,01	6,89	40,0	6,2
	1,50E6	44,39	0,99	8,86		
	1,54E6	43,00	1,01	7,57		
	1,78E6	34,11	1,05	3,51		
	1,54E6	39,30	1,03	4,82		
	1,70E6	37,35	1,03	5,20		
	1,58E6	41,43	1,02	6,31		
	1,52E6	43,84	1,00	8,27		
	1,70E6	37,07	1,04	4,56		

% saturation	Area under the curve		growth rate		mean values of inhibition in %	
	value	inhibition (%)	value	inhibition (%)	AUC	growth rate
26,6	2,57E6	5,00	1,08	0,73	11,2	3,2
	2,27E6	16,09	1,08	0,82		
	2,37E6	12,51	1,09	0,31		
	2,49E6	8,06	1,09	0,15		
	2,41E6	10,84	1,09	-0,00		
	2,18E6	19,28	1,08	0,88		
	2,49E6	8,06	1,10	-0,75		
	2,48E6	9,08	1,10	-0,69		
	2,39E6	11,58	1,08	0,75		
17,6	2,57E6	5,19	1,09	-0,23	2,2	-1,2
	2,77E6	-2,32	1,12	-2,68		
	2,63E6	2,78	1,09	-0,39		
	2,65E6	2,22	1,10	-1,29		
	2,61E6	3,52	1,10	-1,14		
	2,55E6	5,93	1,10	-0,89		
	2,68E6	0,83	1,10	-0,91		
	2,69E6	0,46	1,11	-2,09		
	2,68E6	1,02	1,10	-1,05		
12	2,75E6	-1,58	1,09	-0,31	0,3	0
	2,65E6	2,22	1,09	-0,06		
	2,77E6	-2,41	1,09	-0,54		
	2,70E6	0,28	1,09	0,45		
	2,71E6	-0,19	1,09	0,07		
	2,80E6	-3,34	1,10	-1,14		
	2,61E6	3,71	1,09	0,23		
	2,73E6	-0,74	1,09	0,07		
	2,57E6	4,91	1,08	1,10		

3. Tables for Applicant's Summary and Conclusion

3.1 Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fullfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	X (26)	
Concentration of test substance ≥80% of initial concentration during test		X

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1999e): Toxicity of FAT [redacted] to activated sludge in a respiration inhibition test. [redacted] Report No. [redacted] date: 1999-03-11 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Directive 87/302/EEC, C and OECD No. 209 (1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](DCPP)	
3.1.1	Lot/Batch number	Batch No. [redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	>99%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Stability in water: >1 year (estimated half-life at 25 °C; determined in RCC Project 712260)	x
		Solubility in water: 19.5 mg/L (determined in RCC Project 712012)	
3.1.6	Method of analysis	No chemical analysis of the test substance was performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_4-1	
3.3	**Reference substance**	Yes, 3,5-dichlorophenol	
3.3.1	Method of analysis for reference substance	Reference substance concentrations are not confirmed by analytical method	
3.4	**Testing procedure**		
3.4.1	Culture medium	Synthetic wastewater used:	
		16 g peptone	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

		11 g meat extract 3 g urea 0.7 g NaCl 0.4 g $CaCl_2x2H_2O$ 0.2 g $MgSO_4x7H_2O$ 2.8 g K_2HPO_4 filled up to a final volume of 1 litre with deionised water.
3.4.2	Inoculum / test organism	See table A7_4_1_4-2
3.4.3	Test system	The defined quantity of activated sludge is mixed with synthetic nutrient medium and a respiratory rate is measured after 3 hours. The inhibitory effect of the test item at a particular concentration is expressed as a percentage of the mean of the respiration rates of two controls. An EC_{50} value is calculated from the respiration rates at different test item concentrations.
3.4.4	Test conditions	See table A7_4_1_4-3
3.4.5	Duration of the test	3 hours
3.4.6	Test parameter	Respiration inhibition
3.4.7	Analytical parameter	Oxygen consumption was measured with an oxygen electrode system: WTW OXI 539 meter
3.4.8	Sampling	After exposure time, oxygen consumption was measured. The pH-values and the oxygen concentrations were determined in all test media and the control at the start and at the end of the 3h-incubation period. The water temperature was measured in one control at the start and end of the incubation period and was continuously monitored in all test media and the controls during measurement of the respiration test.
3.4.9	Monitoring of TS concentration	No
3.4.10	Controls	Two controls without test substance are included in the test design. Additionally, a reference substance control was included in the test design.
3.4.11	Statistics	EC_{20}, $EC_{50,}$ EC_{80} were calculated as far as possible by Probit analysis
		4 RESULTS
4.1	**Preliminary test**	Yes
4.1.1	Concentration	Concentrations between 10 and 1000 mg/L were tested
4.1.2	Effect data	Inhibition was between 74-96%
4.2	**Results test substance**	See table A7_4_1_4-4
4.2.1	Initial concentration of test substance	Nominal concentrations: 0.32, 1.0, 3.2, 10, and 32 mg/L
4.2.2	Actual concentrations of test substance	The test substance concentrations are not confirmed by analytical methods.

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

4.2.3	Growth curves	Not reported	
4.2.4	Cell concentration data	Not reported	
4.2.5	Concentration/ response curve	The dose-effect relationship is shown in a graph. Effect values have been plotted against the corresponding concentrations on semi-logarithmic paper.	
4.2.6	Effect data	See table A7_4_1_4-4	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See table A7_4_1_4-4	
4.4	**Test with reference substance**	Performed with 3,5-dichlorophenol	
4.4.1	Concentrations	10, 32 and 100 mg/L	
4.4.2	Results	EC_{50} = 18 mg/L. Thus, the EC_{50} was in the accepted range of 5-30 mg/L and confirms the suitability of the used activated sludge.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The inhibition effect of the test item DCPP on the respiration rate of aerobic wastewater microorganisms of activated sludge was investigated in a 3-hour respiration inhibition test according to the Commission Directive 87/302/EEC, and the OECD Guideline for Testing of Chemicals No. 209. The nominal test concentrations were 0.32, 1.0, 3.2, 10, and 32 mg/L. In addition, two controls and three different concentration of the reference item 3,5-dichlorophenol (10, 32, and 100 mg/L) were tested in parallel.	
5.2	**Results and discussion**	At the lowest concentration of nominal 0.32 mg/L the test item had no significant (<15%) inhibitory effect on the respiration rate of activated sludge after the incubation period of three hours. At the higher test item concentrations of nominal 1.0 and 32 mg/L the inhibitory effect increased to 81% at 32 mg/L.	x
5.2.1	EC_{20}	1 mg/L (95% C.I. = 0.2 – 3 mg/L)	
5.2.2	EC_{50}	8 mg/L (95% C.I. = 3.5 – 28 mg/L)	
5.2.3	EC_{80}	51 mg/L (95% C.I. = 17 – 1048 mg/L)	
5.3	**Conclusion**	DCPP has a significant toxicity towards aquatic bacteria	
5.3.1	Reliability	1	
5.3.2	Deficiencies	-	x

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA VII.7.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2010
Materials and Methods	3.1.5: *Addition*: Vapour pressure: 1.2 x 10^{-6} Pa (at 25°C; determined in RCC Project 711990)
Results and discussion	Agree with applicant's version.
Conclusion	5.2: *Replacement*: Substitute "At the lowest concentration of nominal 0.32 mg/L the test item had no significant (<15%) inhibitory effect on the respiration rate of activated sludge after the incubation period of three hours." by "At the lowest concentration of nominal 0.32 mg/L the test item showed a not significant (<15%) inhibitory effect of 9.1% on the respiration rate of activated sludge after the incubation period of three hours inhibitory effect." 5.3.2: *Addition*: Only for the 2 highest nominal concentrations of 32 and 10 mg/L the amounts of substance were directly weighed into the designated test flasks without using a stock solution. Sulfuric Acid was used to adjust the pH of the activated sludge inoculum.
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Predates recent version of Guideline No. 209, adopted on 22 July 2010: Hence, there are several differences in respect to the updated version. In those details where the test method according to Directive 87/302/EEC differs from OECD No. 209 (1984) the Directive 87/302/EEC was followed.

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_1_4-2: Inoculum/Test organism

Criteria	Details
Nature	Activated sludge
Species	Mixed population of aquatic micro-organisms
Strain	-
Source	Sludge obtained from a domestic wastewater treatment plant (ARA Ergolz II, Füllinsdorf, Switzerland)
Sampling site	Switzerland WWTP
Laboratory culture	No
Method of cultivation	-
Preparation of inoculum for exposure	The sludge was washed by centrifugation, the supernatant liquid was decanted and the solid material resuspended in tap water. This procedure was repeated twice. An aliquot of the final sludge suspension was weighed, thereafter dried and the ratio of wet sludge to its dry weight determined. An aliquot of washed sludge was suspended in tap water to obtain a concentration of 3 g/L.
Pre-treatment	During the holding of three days prior to use, the sludge was fed daily with 50 mL, the sludge was fed daily with 50 mL synthetic wastewater per litre and was kept at room temperature under continuous aeration until use. Immediately before use, the dry weight of the activated sludge was measured again in the inoculum used in the test. The pH of the activated sludge inoculum was adjusted to pH 7.7 with sulphuric acid.
Initial cell concentration	At the start of the test 200 mL activated sludge inoculum with a sludge concentration of 2.4 g/L dry weight were added.

Table A7_4_1_4-3: Test conditions

Criteria	Details
Test temperature	20-21 °C
pH	In the treatment samples: at the start of the test: 8.0 – 8.1 at the end of the test: 9.0
Aeration of dilution water	Yes, with compressed air at a flow of approximately one liter per minute.
Suspended solids concentration	200 mL per test flask

Table A7_4_1_4-4: Influence of DCPP on oxygen consumption of activated sludge

Flask no.	Test chemical	Concentration of test chemical (mg/l)	Oxygen consumption rate (mg O_2/l min^{-1})	Inhibition (%)	pH values		Oxygen concentration (mg O_2/l)	
					start*	end*	start*	end*
1	Control	0	0.588		7.7	8.8	8.3	7.8
10	Control	0	0.554		8.2	8.2	7.5	7.7
Mean Deviation (%)			0.571 5.8					
5	FAT 80'220/A	0.32	0.519	9.1	8.0	9.0	7.5	7.6
6	FAT 80'220/A	1	0.445	22.1	8.0	9.0	7.5	7.6
7	FAT 80'220/A	3.2	0.430	24.7	8.0	9.0	7.5	7.5
8	FAT 80'220/A	10	0.292	48.9	8.0	9.0	7.5	7.6
9	FAT 80'220/A	32	0.109	80.9	8.1	9.0	7.2	8.2

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (2003). Determination of the inhibition of the respiration of activated sludge when exposed to [redacted] Report no. [redacted] Date: 2003-04-01 (unpublished)	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2			
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes OECD Guideline No. 209 (Apr. 1984)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (Methyldiclosan, degradation product of Diclosan)	
3.1.1	Lot/Batch number	[redacted] (expiration date: 31 July 2007)	
3.1.2	Specification	-	
3.1.3	Purity	99.7%	
3.1.4	Composition of Product	Not applicable	
3.1.5	Further relevant properties	- Appearance: white powder - Water solubility: 0.322 ± 0.018 mg/L - Homogeneous and stable, not volatile - Log Pow: 4.58 (estimated)	
3.1.6	Method of analysis	Not performed	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	50,7 mg M-DCPP were mixed with 500 ml deionized water. The mixture was shaken for 24 hours on an orbital shaker. (see table A7_4_1_4-1)	
3.3	**Reference substance**	Yes 3,5-Dichlorophenol ($C_6H_4CI_2O$, CAS-No. 591-35-5)	
3.3.1	Method of analysis for reference substance	Not performed	
3.4	**Testing procedure**		
3.4.1	Culture medium	The nutrient solution was prepared according to guideline. All	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

		chemicals were analytical grade or for use in microbiology. - Peptone: 8.0 g - Meat extract: 5,5g - Urea: 1.5g - NaCl: 0.35 g - $CaCl_2$*2 H_2O: 0.2 g - $MgSO_4$*7 H_2O: 0.1 g - K_2HPO_4: 1.4 g - Deionized water: ad 500 mL	
3.4.2	Inoculum / test organism	Activated sludge from a biologic sewage treatment plant was used. The chosen plant is treating predominantly household sewage. (see table A7_4_1_4-2)	
3.4.3	Test system	See table A7_4_1_4-3	
3.4.4	Test conditions	See table A7_4_1_4-4	
3.4.5	Duration of the test		
3.4.6	Test parameter	Respiration inhibition	
3.4.7	Analytical parameter	Respiration rate (oxygen consumption)	
3.4.8	Sampling	Incubation time: 3 h	
3.4.9	Monitoring of TS concentration	No	
3.4.10	Controls	Control without test substance	
3.4.11	Statistics	The test was performed as a limit test. Therefore no statistical procedures were applied for the test item. The experimental data for the reference item were evaluated by fitting a linear regression curve to the obtained inhibition data.	
		4 RESULTS	
4.1	**Preliminary test**	Not performed	
4.1.1	Concentration	Not applicable	
4.1.2	Effect data	Not applicable	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Stock solution: 100 mg/L (nominal loading, saturated solution)	x
4.2.2	Actual concentrations of test substance	Not determined	
4.2.3	Growth curves	Not applicable	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

4.2.4	Cell concentration data	Not applicable	
4.2.5	Concentration/ response curve	The test was performed as a limit test with one test concentration; therefore a concentration response curve cannot be established.	
4.2.6	Effect data	Inhibition after 3 h: 0.8% 3-h EC50 > 56.8 mg/L (nominal loading) See table A7_4_1_4-5	
4.2.7	Other observed effects	No data	
4.3	**Results of controls**	Positive control: - Mean O_2 consumption = 16.66 mg/(L*h) - Variation 6.2% (recommended limit according to OECD TG No. 209 = 15%) - See table A7_4_1_4-5	
4.4	**Test with reference substance**	Performed	
4.4.1	Concentrations	Nominal: 2.5, 5, 10, 20, 40 mg/L "Real": 2.58, 5.17, 10.33, 20.66, 41.32 mg/L	
4.4.2	Results	3-h EC50 = 13.2 mg/L (95% CL: 11.6 to 15.1 mg/L) This effect value is within the accepted range according to OECD TG No. 209 (5 to 30 g/L). See table A7_4_1_4-5	x
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The toxicity of M-DCPP towards aquatic microorganisms was tested in a respiration inhibition test with activated sludge. The study followed the OECD TG No. 209. The activated sludge was taken from an STP treating predominantly domestic sewage. The test was performed as a limit test. A super-saturated solution of the stock solution was prepared by shaking the test item with deionized water for 24 h. The nominal loading was 100 mg/L. The tested loading was 56.8 mg/L. The oxygen consumption was measured after an incubation period of 3 h. 3,5-Dichlorophenol was used as reference item with a geometric series of 5 concentrations. Analytical monitoring of the tested concentrations was not performed.	x
5.2	**Results and discussion**	The test solution was prepared as a super-saturated solution with a loading of 56.8 mg/L. No chemical analysis of the test solution was performed as this is not required according to the guideline. No significant inhibiting effect could be observed on the respiration activity of activated sludge. The 3-h EC20 was determined to be > 56.8 mg/L.	
5.2.1	EC_{20}	> 56.8 mg/L (nominal loading); > water solubility	
5.2.2	EC_{50}	> 56.8 mg/L (nominal loading) ; > water solubility	
5.2.3	EC_{80}	> 56.8 mg/L (nominal loading) ; > water solubility	

Section A7.4.1.4 Inhibition to microbial activity (aquatic)

Annex Point IIA7.4

5.3	**Conclusion**	The validity criteria were met (See table A7_4_1_4-6).	x
5.3.1	Reliability	2	
5.3.2	Deficiencies	Yes - In Ch. 9.2 of the study report the test concentrations of reference and test item are listed as “nominal” and “real”. Since no chemical analysis was performed, the meaning of “real” is unclear.	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	September 2013
Materials and Methods	4.2.1 *Correction*: Concentration of the Stock soluation was 101.4 mg/L (nominal). The concentration of the test item in the test vessels were 56.8 mg/L (nominal). 4.4.2 *Correction:* For total respiration the EC_{50} of 3,5-DCP lies between 2 and 25 **m**g/L according to OECD 209 (2010).
Results and discussion	Agree with the applicant´s version.
Conclusion	No inhibition is observed at the highest test concentration, therefore the NOEC is set equal to the water solubility of 322 ± 18 µg/L.
Reliability	2
Acceptability	Acceptable
Remarks	-

Table A7_4_1_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	Not applicable
Vehicle control performed	Not applicable
Other procedures	Test item was shaken for 24 h in deionized water on an orbital shaker (50.7 mg test item in 500 mL deionized water).

Table A7_4_1_4-2: Inoculum / Test organism

Criteria	Details
Nature	activated sludge
Source	sewage treatment plant treating predominantly domestic sewage
Sampling site	Activation basin of the ESN (Stadtentsorgung [redacted]) sewage treatment plant, [redacted] Sampling date: 21 Nov 2002
Laboratory culture	No
Method of cultivation	Not applicable
Preparation of inoculum for exposure	The sludge was washed with tap water three times, then centrifuged and re-suspended in tap water.
Pretreatment	No
Initial cell concentration	The dry matter was determined as 3620 mg suspended solids/L. The pH was 7.7.

Table A7_4_1_4-3: Test system

Criteria	Details
Culturing apparatus	Glass beakers, fill volume: approx. 500 mL
Number of culture flasks/concentration	2 per treatment and 4 per control
Aeration device	Purified air, using Pasteur pipettes
Measuring equipment	- O_2-electrode (oxygen meter: Oxi 538 wtw) - Flat-bottom narrow neck glass flask (no headspace)
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_1_4-4: Test conditions

Criteria	Details
Test temperature	21±1 °C
pH	No data
Aeration of dilution water	Not specified. Inoculum was aerated. Flow: approx. 0.75 L/min
Suspended solids concentration	No data

Table A7_4_1_4-5: Oxygen consumption (mean values) and respiration inhibition

Sample	Nominal conc. (mg/L)	Oxygen consumption (mg/(l*h)	Respiration inhibition (%)
Blank	0	16.13	-
Reference item	2.5	14.52	12.85
	5	13.00	32.96
	10	9.44	43.32
	20	6.26	62.43
	40	3.20	80.78
Test item	56.8	16.52	0.80
Blank	0	17.19	-

Table A7_4_2_1-6: Validity criteria for "activated sludge, respiration inhibition test" according to OECD Guideline 209 (1984)

	fulfilled	Not fulfilled
Control respiration rates are within 15% of each other	**X**	
3-h EC50 of reference substance (3,5-dichlororphenol) within 5 to 30 mg/L	**X**	

Section 7.4.3.2 Annex Point IIIA XIII.2.2	Effects on reproduction and growth rate of fish	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [ ]** **Other justification [X].**	 x
Detailed justification:	DCPP is intended to be used as disinfectant in products belonging to Biocidal Product Types (PT) 1, 2 and 4. Long-term aquatic fish toxicity is a test requirement for active substances used in PT 2, unless the release is intermittent or the intended use is limited to closed spaces with insignificant aquatic release. As both restrictions are fulfilled for the indoor use of DCPP as disinfectant of hard surfaces, no long-term tests on aquatic organisms are required. For active substances used as PT 1 and 4, no aquatic long-term toxicity tests are requested at all. However, a higher tier early life stage test on fish is available with the structurally similar active substance triclosan (CAS-No. 3380-34-5). The phenol ring of triclosan and DCPP is identical. Furthermore, since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that triclosan has a higher potential for bioaccumulation and a higher toxicity to organisms than DCPP. As expected, comparing the results of the acute toxicity tests, triclosan showed a higher toxicity than DCPP (LC_{50} of 0.09 mg/L vs. 0.7 mg/L). Thus, the long-term toxicity of DCPP is also expected to be lower than the one of triclosan indicating that the results obtained in the study with triclosan cover effects of long-term exposure of fish to DCPP.	x x x
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	April 2011	
Evaluation of applicant's justification	Delete cross at “Other justification”. Insert “regarding effects on reproduction and the growth rate on an appropriate species of fish ”after term “Long-term aquatic fish toxicity”. *Deletion:* As both restrictions are fulfilled for the indoor use of DCPP as disinfectant of hard surfaces, no long-term tests on aquatic organisms are required. Insert “0.52 mg/l” instead of “0.09 mg/L”.	
Conclusion	Agree with applicant’s version with amendments given above.	
Remarks	-	

Section 7.4.3.2 Effects on reproduction and growth rate of fish

Annex Point IIIA XIII.2.2

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1996): Early Life-Stage Toxicity of Triclosan to the rainbow trout (*Oncorhynchus mykiss*) under flow-through conditions. [redacted] Report No. [redacted] date: 1996-11-27 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	BASF SE	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	U.S. EPA-FIFRA, 40 CFR, Section 158.145, Guideline 72-4 Similar to OECD 210 (1992) and U.S. EPA Pesticides Assessment Guideline for fish early life-stage (1986)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	**Triclosan**	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	-	
3.1.3	Purity	99.5%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Water solubility: 0.012 g/L at 20 °C (Pointurier, 1990; cf. section 2 of III-A)	x
3.1.6	Method of analysis	HPLC; fortification concentrations for the method validation ranged from 3.00 to 300 µg/L and yielded a mean recovery of 96.3 ± 2.3%.	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Stock solution was prepared by dissolving 0.662 g of Triclosan in 100 mL of DMF in a volumetric flask. Stock solutions were placed in an amber bottle which fed the syringe injector. See also table A7_4_3_2-1.	x
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_2-2	
3.4.2	Test organisms	*Oncorhynchus mykiss*, see table A7_4_3_2-3	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII.2.2

3.4.3	Handling of embryos and larvae (OECD 210/212)	See table A7_4_3_2-3 and -4	
3.4.4	Test system	See table A7_4_3_2-4	x
3.4.5	Test conditions	See table A7_4_3_2-5	
3.4.6	Duration of the test	Exposure time: 96 days (61 post-hatching)	
3.4.7	Test parameter(s)	Biological parameters: - Developing embryos were observed daily for mortality (change in coloration). When hatch began, the number of embryos hatched in each incubation cup was recorded daily until hatch was complete. The 61 day-post-hatch growth period began when 95% of the eggs in the control and vehicle blank had hatched. - Fry-observations of abnormal behaviour, abnormal swim-up behaviour, abnormal physical change, and mortality were recorded daily by visual inspection of each growth chamber. Chemical and physical parameters: Temperature, dissolved oxygen, pH-value, conductivity, hardness, and alkalinity were measured and recorded on Days 0, 1 (except for hardness and alkalinity), 7, and weekly thereafter, including study determination.	
3.4.8	Examination / Sampling	Fry growth as measured by standard length (mm), was determined by using a photographic method similar to that described by McKim and Benoit (1971) on Day 35 post-hatch. At study termination all surviving fish were sacrificed and individually measured for standard length. After being measured for length, each fish was weighed.	
3.4.9	Monitoring of TS concentration	Yes, at study Days 5, 0, 1, 7 and at least weekly thereafter from composite samples of two of the four replicate chambers of the control, DMF, vehicle blank, and each test concentration.	x x
3.4.10	Statistics	Survival and hatchability data within treatment differences were analysed using a one-tailed Fischer's exact test and simple chi-square statistic. Growth data was analysed using ANOVA, followed by a one-tailed Dunnett's multiple comparison procedure to assess differences between the control and each of the Triclosan test concentrations. The dilution water control and DMF vehicle blank were compared using a least squares means procedure to determine whether or not they could be pooled. All statistical analysis were performed using SAS for Windows, Release 6.10. Inferences of statistical significance were based upon a $p \leq 0.05$.	
		4 RESULTS	
4.1	**Range finding test**		
4.1.1	Concentrations	A 46-days range finding study with Triclosan was conducted under flow-through conditions in order to estimate the nominal range for the definitive study. Two replicates per treatment each with 20 lightly eyed rainbow trout embryos were exposed to dilution water control, DMF	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII.2.2

		vehicle blank (0.0125 mL/L), 0.033, 0.065, 0.13, 0.25, and 0.50 mg a.s./L. Endpoints examined: embryo hatchability, fry survival, growth, swim-up behaviour, and physical an behavioural abnormalities.	
4.1.2	Number/ percentage of animals showing adverse effects	Embryos began hatching on Day 13. Mortality on Day 46 was 95, 100 and 100% at the three highest concentrations respectively. Abnormal behaviour was observed predominately at almost every concentration. Based on length measurements significant reduction was observed at 0.065 mg/L. Regarding wet weight no effects were given.	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 5.0, 10, 20, 40, and 80 µg a.s./L. Additionally, a dilution water control and DMF vehicle blank were run.	
4.2.2	Actual concentrations of test substance	Mean measured concentration of Triclosan determined via HPLC were < 1.31(control), < 1.31 (DMF vehicle blank), 4.26, 8.63, 17.1, 34.1, and 71.3 µg a.s./L. These values ranged from 85 to 89% of the nominal test concentrations which were 5.0, 10, 20, 40, and 80 µg a.s./L.	
4.2.3	Effect data	Confer table A7_4_3_2-6	
4.2.4	Concentration / response curve	Yes, several diagrams are included in the report for each endpoint	
4.2.5	Other effects	-	
4.3	**Results of controls**		
4.3.1	Number/ percentage of animals showing adverse effects	Concerning the mortality and abnormal behaviour in the vehicle blank and dilution water control, results were in the validity range.	x
4.3.2	Nature of adverse effects	Non-treatment control presented 3% abnormalities over the study period which were related to spinal curvatures	x
4.4	**Test with reference substance**		
4.4.1	Concentrations	-	
4.4.2	Results	-	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII.2.2

		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A flow-through early life-stage toxicity study was conducted with rainbow trout exposed to a dilution water control, DMF vehicle blank, and five concentrations of Triclosan to determine the maximum acceptable toxicant concentration (MATC) limits (geometric mean between NOEC and LOEC). This was achieved by measuring the effects of exposure of Triclosan on egg hatchability, fry survival, and fry growth and by noting any abnormal physical change in the eggs and post-hatch fry as determined by comparing control and exposure concentrations. Newly fertilised eggs (< 3.5 hours post-fertilisation) were placed in exposure chamber to initiate the study and exposure continued for a total of 96 days (61 days post-hatch). An intermittent flow 2-L proportional diluter system as used to maintain constant test concentrations. Mean measured concentration of Triclosan determined by HPLC were 0.0 (control), 0.0 (DMF vehicle blank), 4.26, 8.63, 17.1, 34.1, and 71.3 µg a.s./L. These values ranged from 85 to 89% of the nominal test concentrations which were dilution water control, DMF vehicle blank, 5.0, 10, 20, 40, and 80 µg a.s./L	
5.2	**Results and discussion**	Egg hatchability was not affected at any test concentration when compared to the pooled control group. Survival at 35 days was significantly reduced (p=0.00) at 71.3 µg/L (level 5) when compared to the pooled control group. Survival at 61 days post-hatch was significantly reduced (p=0.00) in the 71.3 µg/L (level 5) treatment compared to the pooled control group. At 35 days post-hatch, mean standard length was not significantly reduced in any test concentration when compared to the pooled control group. At 61 days post-hatch, both mean standard length and dry weight were not significantly reduced in any test concentration when compared to the pooled control group. Time to 95% hatch was not affected at any test concentration when compared to the pooled control group. Compared to the pooled control group, time of initiation of sustained swim-up behaviour was delayed at 71.3 µg a.s./L. Compound-related morphological and behavioural effects were not observed in any test concentration.	
5.2.1	NOEC	NOEC = 34.1 µg a.s./L, based on mean measured conc.	x
5.2.2	LOEC	LOEC = 71.3 µg a.s./L, based on mean measured conc.	x
5.3	**Conclusion**	Egg hatchability was not affected at any Triclosan concentration tested. Fry survival was significantly reduced at a concentration of 71.3 µg a.s./L. Growth, as measured by standard length and dry weight, exhibited no reduction at any Triclosan concentration tested.	x
		Based on the most sensitive endpoint (i.e. survival) evaluated during the study, the geometric mean-maximum acceptable toxicant concentration (GM-MATC) for Triclosan is estimated to be 49.3 µg a.s./L. Based on the lack of hatch and growth effects, the NOEC and LOEC are 34.1 and 71.3 µg a.s./L, respectively.	x x
		The test is considered to be valid. For validity criteria according to OECD Guidelines 210/212 see Table A7_4_3_2-7.	
5.3.1	Other Conclusions	-	

Section 7.4.3.2 Effects on reproduction and growth rate of fish
Annex Point IIIA XIII.2.2

5.3.2	Reliability	1
5.3.3	Deficiencies	No

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	April 2011
Materials and Methods	3.1.5: No information on vapour pressure provided. 3.2: Insert “0.622” instead of “0.662”. 3.4.4: ad Table A7_4_3_2-4: In box for “Volume of test vessels” add “,yielding a test volume solution 9.94 L.” following the term “(305×205×210 mm)”. Delete sentence “Test solution volume was nominally 9.94 L.” in box for “Volume/animal”. 3.4.5: ad Table A7_4_3_2-5: Add “(mean % of saturation)” after “10°C”. 3.4.9: Insert “-1, 0, 5, 7” instead of “5, 0, 1, 7”. 3.4.9: Delete “,” after “DMF”.
Results and discussion	4.1.1: Add “d” to term “an”. 4.2.3: ad Table A7_4_3_2-6: Insert “98.2” instead of “92.2” in the box describing 61-day post hatch survival (%) for dilution water control. Add “(mean % of saturation)” after “10°C”. 4.3.1: Remove “Concerning the mortality and abnormal behaviour in the vehicle blank and dilution water control, results were in the validity range.” by “The mortality in the control and vehicle control were 1.8% and 1.7 % at 61 days post hatching.” 4.3.2: Results for behavioural and morphological abnormalities in the final test are not presented in the original study report. Only data for the range finding test is presented. It is therefore unclear whether the 3 % abnormalities in the control (spinal curvatures) refer to the range finding test or the final test.
Conclusion	Adopt applicant´s version.
Reliability	1-2
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_4_3_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, DMF solvent
Concentration of vehicle	max. 0.013 mL/L (0.00081, 0.0016, 0.0033, 0.0065, and 0.013 mL/L for levels 1-5, respectively)
Vehicle control performed	Yes, 0.013 mL/L DMF
Other procedures	No

Table A7_4_3_2-2: Dilution water

Criteria	Details
Source	Deep well water passed through a reverse osmosis system and then blended with well water.
Alkalinity ($CaCO_3$)	130 to 158 mg/L
Hardness ($CaCO_3$)	128 to 152 mg/L (as $CaCO_3$)
pH	7.92 - 8.35
Oxygen content	9.4 to 9.7 mg/L (mean of samples over the test period per treatment), always above 85% oxygen saturation limit at 10°C
Conductivity	322 to 455 µS
TOC Content	< 1.0 mg/L
Holding water different from dilution water	Fertilised eggs were immediately covered with dilution water

Table A7_4_3_2-3: Test organisms

Criteria	Details
Species	Rainbow trout (*Oncorhynchus mykiss*)
Source	[redacted]
Wild caught	No
Age/size	Developing embryos at the start of the test
Kind of food	Brine shrimp nauplii (*Artemia* sp.) Salmon starter mash Salmon starter pellet
Amount of food	*Ad libitum*
Feeding frequency	Sac-fry fed were provided a live brine shrimp nauplii (*Artemia* sp.) diet from Day 49 through Day 94. A commercially prepared salmon starter was added to the diet on Day 61. Salmon starter pellet size was increased as the fry increased in size. Trout were fed *ad libitum* at least three times a day during the week and at least twice a day on weekends and holidays.
Post-hatch transfer time	The sac-fry were released unharmed into the growth chamber on Day 36
Time to first feeding	Food was introduced on Day 49 (14 days post-hatch) as the sac-fry began to exhibit swim-up behaviour.
Feeding of animals during test	Yes
Treatment for disease within 2 weeks proceeding test	No

Table A7_4_3_2-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	During the study, control dilution water and test solution were delivered to each replicate chamber at a mean rate of 82 L/replicate/day, a rate sufficient to replace the 9.94 L volume an average of 8.25 times in a 24-hour period. As the study progressed, flow rate was increased to maintain water quality and reduce biomass loading. During the last 14 days of the study, a mean flow rate of 133 L/replicate/day provided 13.4 volume replacements in a 24-hour period. At this flow rate, a biomass loading of 0.194 g/L/day at study termination was calculated in replicate B of the control.
Volume of test vessels	Developing embryos were incubated in glass cups constructed from 9-cm diameter flint jars with the bottoms removed and replaced with 16-mesh Nitex screen attached with silicon adhesive. The incubation cups were suspended with stainless steel wire in each replicate chamber. For both egg hatching and growth chambers, replicates from plate glass glued together with silicone adhesive were used. Individual chambers measured 15.4 cm wide x 30.6 cm long x 21.1 cm water depth (305×205×210 mm).
Volume/animal	Test solution volume was nominally 9.94 L
Number of animals/vessel	40 eggs/incubation cup An additional 50 eggs, were transferred into each of the four replicates of the dilution water control chambers to measure fertilisation success on Day 12
Number of vessels/ concentration	4 replicates per solvent control/dilution water control/test concentration
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_2-5: Test conditions

Criteria	Details
Test temperature	10.3 to 10.5 °C (mean temperatures of two replicates/treatment)
Dissolved oxygen	> 85% oxygen saturation limit at 10°C
pH	8.17 to 8.21(mean values on every given day)
Adjustment of pH	No
Aeration of dilution water	No data
Intensity of irradiation	Light intensity: 59.4 ± 6.24 footcandles (measured on Day 48 and 84)
Photoperiod	Developing embryos were maintained in the dark until one week after hatch was complete. Beginning on Day 42 (Day 7 post-hatch) all aquaria were illuminated 100% intensity on a 16-hour light: 8-hour dark photoperiod preceded by a 30-min simulated dawn and dusk transition period. Illumination was provided by wide-spectrum fluorescence bulbs and plant grow lights (CRI>90)

Table A7_4_3_2-6: Time to 95% embryo hatch and time to initiation of swim-up behaviour, egg hatchability and survival of rainbow trout exposed to Triclosan (mean values of 4 replicates each)

Mean measured Triclosan concentration (µg a.s./L)	Time to 95% hatch (days)	Time to initiation of sustained swim-up (days)	Egg hatch (%)	35-day post-hatch survival (%)	61-day post-hatch survival (%)
Dilution water control	35	48	91.7	100	92.2
DMF vehicle blank	35	49	94.4	100	98.3
Pooled control	35	49	93.0	100	98.3
4.26	35	49	93.7	98.3	98.3
8.63	35	50	95.8	100	98.3
17.1	35	49	95.2	98.3	95.0
34.1	35	50	90.3	100	98.2
71.3	35	54	95.8	56.7	56.7

Table A7_4_3_2-7: Validity criteria for an ELS fish test according to OECD Guidelines 210

	fulfilled	Not fulfilled
Concentration of dissolved oxygen > 60% saturation throughout the test	**X**	
Difference of water temperature < 1.5% between test chambers or successive days at any time during test; temperature within range for specific test species	**X**	
Overall survival of fertilized eggs in controls (and solvent controls) ≥ value, specified for the specific test species	**X**	
Test substance concentrations maintained within ± 20% of mean measured values	**X**	
No effect on survival nor any other adverse effect found in solvent control	**X**	
Further criteria for poorly soluble test substances	**X**	

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2000): Bioconcentration test of [redacted] in carp (*Cyprinus carpio*). [redacted] Report No. [redacted] CR, date: 2000-05-08 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	'Bioconcentration: Flow-through Fish Test", OECD Guideline for Testing of Chemicals, No. 305, June 14, 1996; 'Method for Testing the degree of Accumulation of Chemical Substance in Fish Body" stipulated in the 'Testing Methods for New Chemical Substances" (July 13, 1974, Kanpogyo No.5, Planning and Coordination Bureau, Environment Agency, Yakuhatu No. 615, Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, and 49 Kikyoku No. 392, Basic Industries Bureau, Ministry of International Trade and Industry, Japan).	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](2-Hydroxy-4, 4'-dichlorodiphenylether = DCPP)	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	99%	
3.1.4	Further relevant properties	Water solubility: 19.5 mg a.s./L	
3.1.5	Radiolabelling	None	
3.1.6	Method of analysis	HPLC-UV	
3.2	**Reference substance**	No	
3.2.1	Method of analysis for reference substance	-	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	Acute toxicity test with *brachydanio rerio* Acute toxicity test in *brachydanio rerio* for concentration selection of the	

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

test substance (LC_{50} study for 96 hours) (SOP 2.6)	
This study was performed in accordance with OECD guideline No. 203. Test fish was the species *brachydanio rerio* (supplier [redacted]; 2-week acclimatisation phase; mean body length: 3.2 cm; mean body weight : 0.24 g. Each test solution was obtained from the diluted stock solution with fish breeding water; at 23.5°C; semi-static system (renewal every 48 hours), 10 fish/4L test medium	
LC_{50} = 0.86 mg/L, based on measured results (HPLC analytical monitoring)	x
Test animals	
Species Carp, *Cyprinus carpio L.* was supplied by [redacted]	
The acclimatisation was of nearly 4 months and additionally 7 days in the test system.	
The fish were examined for health during 7 days of acclimation period. The selected carp had a mean body weight of 6.44± 0.81 g and mean body length of 7.8± 0.4 cm.	x
Diet Pelleted standard diet for carp (Suimii) was used (crude protein > 37.0%, crude fat >2.5%, obtained from Japan Pet Food Ltd. The diet was provided with about 2% of body weight and was not provided the day before sampling.	
Stock solution	
The concentration of test substance into distilled pure water was 20 mg/mL. Each test solution (level 1 and 2) was obtained from the diluted stock solution with fish breeding water.	x
Level 1: The stock solution was diluted with fish breeding water and 2.0 mg/mL test solution was prepared. The test solution was further diluted with test water, and then added continuously to glass tank.	
Level 2: The stock solution was diluted with fish breeding water and 0.2 mg/mL test solution was prepared. The test solution was further diluted with test water, and then added continuously to glass tank.	
Control: fish breeding water was added.	
Test system	
The test was run under flow-through conditions (flow rate: 300 mL/min or 432 L/day). The glass tanks had a volume of 100 L. Level 1, Level 2 and control were used test tank No.4, No.5 and No.6, respectively.	x
Test concentrations were 0.02 mg/L and 0.002 mg/L and blank control. Only one replicate.	x
The test water consisted in fish breeding water, hardness 84 mg/L, pH 7.3, oxygen content 7.6 ± 0.1 mg/L, temperature: 25 ± 0.1°C, pH: 7.3± 0.1, 10 hour light/14 hours darkness (dim light: fluorescent lamp).	x
The duration of the test was in total 4 weeks for the bioaccumulation period and 1 week for the depuration period.	
38 fish were sued for each level tested and for the control 10 fish.	

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

		Analysis	
		The analysis of the test water and the test fish (two fish each series) for accumulation were performed after 7, 14, 21, 26, and 28 days, additionally test water was analysed at test initiation. In the control test fish were only measured at the end of the test. Three days and 7 days after the start of the depuration phase, test fish were analysed.	x
		The measurement of water quality parameters (pH, temperature, oxygen) were recorded at the day of water analysis.	x
3.3.2	Estimation of bioconcentration	Calculation of results	
		In evaluating the data obtained from the bioconcentration study, a steady-state approach was used. This consists of a two compartment model (water and fish) which is used to describe the movement of the test material in and out of the test fish. This approach is used to determine the steady-state bioconcentration factor (BCF).	
		$\text{Bioconcentration factor} = \frac{C_F}{C_W}$	
		C_F = Mean concentration of the test substance in test fish under equilibrium C_W = Mean concentration of the test substance in the test tank under equilibrium	
		It was judged as the equilibrium when the standard deviation of BCF was within mean ± 20% at continuous measurement of 3 point for beyond 48 hours.	
		In the results of the test fish analysis in control at 0 (n=1) day and 28 days (n=1) from start of exposure, the background in peak position of test substance was less than minimum determination limit of the test substance.	
		4 RESULTS	
4.1	**Experimental data**		
4.1.1	Mortality/behaviour	No mortality occurred throughout the test.	
4.1.2	Lipid content	Mean Lipid Content (n=2), measured values by $CHC1_3$ - MeOH method:	
		Pre-exposure: 3.2% Termination: 3.6%	
4.1.3	Concentrations of test material during test	See table A7_4_3_3_1-1	
4.1.4	Bioconcentration factor (BCF)	Mean concentrations and standard deviations of BCFs Level 1 : BCF = 67.4 ± 8.9 Level 2 : BCF = 76.7 ± 6.6	
		See also table A7_4_3_3_1-2.	x
4.1.5	Uptake and depuration rate constants	Not measured	

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

4.1.6	Depuration time	More than 95% of the amount of test substance residual in corp was eliminated in 7 days in Level 1 and 2. See also table A7_4_3_3_1-3.
4.1.7	Metabolites	No metabolites identified
4.1.8	Other Observations	-
4.2	**Estimation of bioconcentration**	Bioconcentration factor is based on measurements.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Bioconcentration test of DCPP in carp was performed in accordance with OECD guideline 305 and Japanese standard “Testing Methods for New Chemical Substances” (July 13, 1974, Ministry of International Trade and Industry, Japan). The concentrations of the test substance were selected to be 0.02 mg/L and 0.002 mg/L based on an acute toxicity screening test (LC_{50} value for 96 hours in *Brachydanio rerio* was 0.86 mg/L). Bioconcentration test was performed for 28 days because equilibrium was reached at 7 days after the start of uptake phase. After the end of exposure period, the depuration phase was performed. The results of Bioconcentration factors of the test substance in Level 1 (0.02 mg/L) and Level 2 (0.002 mg/L) were calculated from 51 to 84 times and from 69 to 89 times, respectively. Therefore, the steady-state bioconcentration factors (BCFss) in Level 1 and Level 2 under the equilibrium were calculated to 68 times and 77 times, respectively.
5.2	**Results and discussion**	The BCF for level 1 and 2 were 67.4 ± 8.9 and 76.7 ± 6.6, respectively. During the depuration phase more than 95% of the amount of test substance residual in corp was eliminated within 7 days in Level 1 and 2.
5.3	**Conclusion**	It is concluded that the test substance, DCPP, had low accumulation in carp and had rapid elimination from carp under this test conditions.
5.3.1	Reliability	1
5.3.2	Deficiencies	Uptake and depuration rates are missing

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	December 2010
Materials and Methods	3.3.1: ad Acute toxicity test with *Brachydanio rerio:* Replace phrase "based on measured results (HPLC analytical monitoring) by "based on initial concentration levels measured by HPLC" 3.3.1: ad test animal: No information on age or sex of the fish is provided, neither whether fish derive from the same year class. 3.3.1: ad dilution water used for screening pre-test and the bioconcentration test: suspended solids were not measured as prescribed according to OECD guideline No. 305; no analysis regarding several heavy metals (Pb, Ni), total organochlorine pesticides and the major ions like Ca, MG, Na, K and SO4 was conducted. 3.3.1: Less than the preferred minimum five volume replacements per day were achieved. 3.3.1: Replace bullet "The test water consisted in fish breeding water, hardness 84 mg/L, pH 7.3, oxygen content 7.6 ± 0.1 mg/L, temperature: 25 ± 0.1°C, pH: 7.3± 0.1, 10 hour light/14 hours darkness (dim light: fluorescent lamp)" by "The water used for the preparation of the test media was characterised having a hardness of 84 mg/L, pH of 7.3 and TOC < 2 mg/L. During the test (measured at least once a week), the pH ranged from 7.0 to 7.3 in the control and 7.2 to 7.4 in the two test media containing DCPP. The oxygen content was 7.6 in the control and ranged from 7.4 to 7.6 in the two test media containing DCPP. The temperature in the control and the two test media containing DCPP ranged from 25.1 to 25.5°C. For the lighting dim light (fluorescent lamp) with 10 hours light/14 hours darkness was used." 3.3.1: ad lighting: The photoperiod of 10 hours differs from the usually deemed appropriate period of 12 to 16 hours according to according to OECD guideline No. 305. 3.3.1: ad depuration phase: Sampling only occurred on 2 occasions in the depurination phase, while four are prescribed by OECD guideline No. 305. 3.3.1: ad water quality parameters: No TOC or hardness measurements took place during the test, although demanded by OECD guideline No. 305; temperature was not measured daily.
Results and discussion	4.1.4: *Addition*: The sample size per each sampling time per level was only three fish – of these two were used for BCF calculation.
Conclusion	5.3.2: *Addition*: See statements given above.

Section A7.4.3.3.1 Bioconcentration in aquatic organisms (fish)

Annex Point IIIA, XIII.2.3 *Cyprinus carpio*

Reliability	2, as the study has some considerable flaws as mentioned above. Particularly, no TOC or suspended solids measurement during the testing took place which could lead to an underestimation of BCF due to adsorption of DCCP. Nevertheless, analytical data provided demonstrated stable DCPP concentration during the test. Moreover, the fish tested at each concentration should have been four instead of two according to OECD guideline No. 305 and the interval for the sampling could have been shorter. Nevertheless, 5 measurements took place supporting the steady state concentration over a period of time.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_4_3_3_1-1: Concentration of the test substance in test water

	Day 0	Day 7	Day 14	Day 21	Day 26	Day 28
Level 1	0.0178	0.0180	0.0199	0.0188	0.0209	0.0199
Level 2	0.00183	0.00211	0.00185	0.00187	0.00195	0.00208

Table A7_4_3_3_1-2: Bioconcentration factors

The Day from start of exposure	Level 1		Level 2	
	Sample number (n=2)	BCFs	Sample number (n=2)	BCFs
7	No.1	78	No.1	69
	No.2	84	No.2	73
14	No.1	51	No.1	73
	No.2	58	No.2	70
21	No.1	68	No.1	76
	No.2	70	No.2	76
26	No.1	69	No.1	89
	No.2	65	No.2	89
28	No.1	69	No.1	77
	No.2	62	No.2	75

Table A7_4_3_3_1-3: Depuration results

Days	Level 1			Level 2		
	Sample No. (n=2)	Residual Rate (%)	Concentration in Fish (µg/g)	Sample No. (n=2)	Residual Rate (%)	Concentration in Fish (µg/g)
3	No.1	2	0.0294	No.1	10	0.0154
	No.2	2	0.0229	No.2	11	0.0159
7	No.1	2	0.0230	No.1	<5	<0.00771
	No.2	1	0.0115	No.2	<5	<0.00807

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

Official use only

	1 REFERENCE	
1.1	**Reference**	[redacted]. Determination of bioconcentration of [redacted] in the flow-through fish test, using the species *Danio rerio*. [redacted] Report no. [redacted] Date: 2003-06-24 (unpublished)
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA
	2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes - OECD Guideline No. 305 (June 1996) - EU Method C.13 (Bioconcentration: Flow-through fish test)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes - No concentration given for solvent (methanol). - No solvent control performed. - Test concentration in lower treatment level was not maintained within ±20% of the mean concentration.
	3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted](Methyldiclosan, degradation product of Diclosan)
3.1.1	Lot/Batch number	[redacted] (expiration date: 31 July 2007)
3.1.2	Specification	-
3.1.3	Purity	99.7%
3.1.4	Further relevant properties	- Appearance: white powder - Water solubility: 0.322 ± 0.018 mg/L - Homogeneous and stable, not volatile - Log Pow: 4.58 (estimated) - Fish: 96-h LC50 > limit of water solubility (> 320 µg/L)
3.1.5	Radiolabelling	No
3.1.6	Method of analysis	Gas chromatography - Solvent: iso-octane - Internal standard: hexachlorobenzene

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

		- Gas chromatograph: GC 6890 A, HP, Ser.-No. US00000507 - Column: BGB 5, 30 m, 0.25 mm, 0.25 µm - GC method: 02080201G - Calibrated sector of method: 5 – 50 mg/L - R of calibration function: 0.9997 - Standard deviation of injection: 3.6% - Recovery rate (water) at 10%: 96 ± 3% - Recovery rate (water) at 90%: 97 ± 3% - Validation report: VB02080201G926	
3.2	**Reference substance**	No (not required)	
3.2.1	Method of analysis for reference substance	Not applicable	
3.3	**Testing/estimation procedure**		
3.3.1	Test system/ performance	- Vessels: Glass aquaria - Photoperiod: 12/12 hours, using fluorescent tubes - Temperature: 23 ± 2 °C - Feeding: warm-water fish food (flakes and tablets were given), 1 - 2% of body weight/day; recalculation of the amount needed was performed once a week on the basis of the weight of the most recently sampled fish - Cleaning: approx. 30 minutes after feeding by siphoning the bottom of each chamber to remove debris and uneaten food - Flow check: daily - Pump calibration: once per week - Aeration: purified air was conducted into the test chambers - Uptake period: 19 d - Depuration period: 18 d	x, x, x
3.3.2	Estimation of bioconcentration	The bioconcentration was not estimated within the study report. The log Pow is estimated to be 4.58 using the KOWWIN module v1.68 of EPI Suite v4.10 (US EPA, 2012). This value indicates that accumulation in organisms is possible.	
		4 RESULTS	
4.1	**Experimental data**		

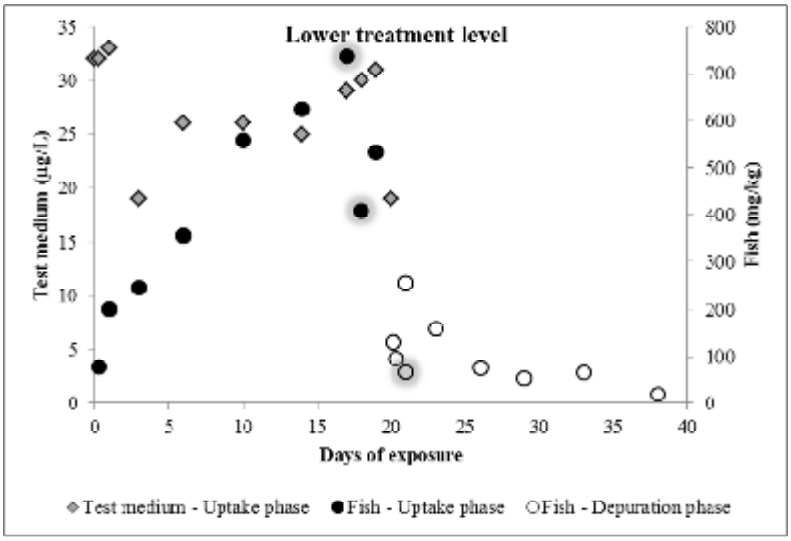
Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

4.1.1	Mortality/behaviour	- Mortality: 0% - Behaviour: Fish in upper treatment level did not eat and showed slackened movements during uptake period. Food consumption and agility was normal during acclimatisation and depuration period.	
4.1.2	Lipid content	See Tables A7_4_2_1-6 (uptake period) and A7_4_2_1-7 (depuration period)	x

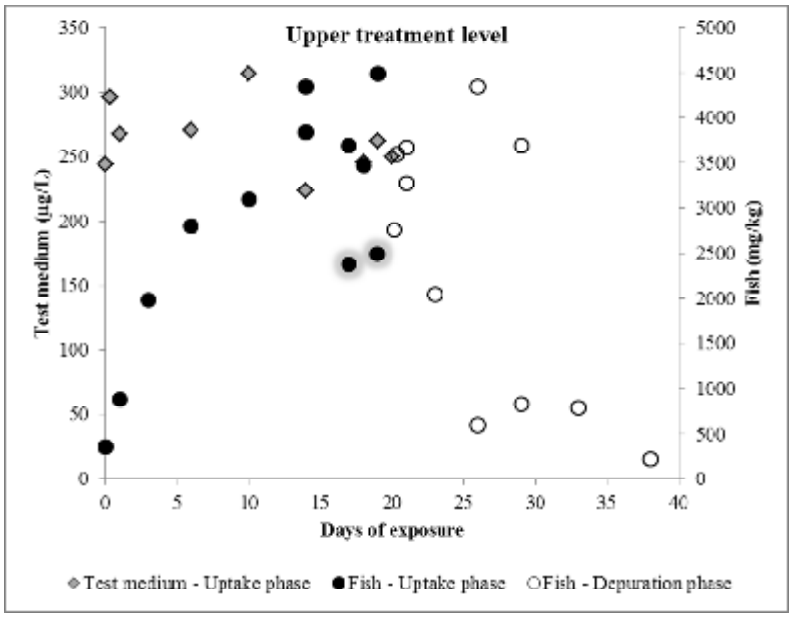
Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

4.1.3	Concentrations of test material during test	See Tables A7_4_2_1-6 (uptake period) and A7_4_2_1-7 (depuration period) **Control:** M-DCPP was not found in the test medium or the fish **Lower treatment level** (Figure see below, outliers marked by grey outer circle): - Test medium (uptake phase): 27.5±5.0 µg/L (range: 19 to 33 µg/L) - Fish: plateau phase (start: day 10): 572 ± 120 mg/kg (range: 409 to 736 mg/kg; based on all values)  **Upper treatment level** (Figure see below, outliers marked by grey outer circle): - Test medium (uptake phase): 263±28 µg/L (range: 223 to 314 µg/L) - Fish: plateau phase (start: day 10): 3769 ± 541 mg/kg (range: 3094 to 4499 mg/kg; without outliers)	x, x

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5



Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

4.1.4	Bioconcentration factor (BCF)	**Lower treatment level** - $BCF_{ss} = C_{fish\_ss}/C_w$ = 572 mg/kg / 27.5 µg/L = 20800 L/kg (recalculated; BCF_{ss} value from report: 21285 L/kg) - BCF_k: The kinetic constants were determined according to three methods. For results see Table A7_4_2_1-7. Since k1, k2 and BCF derived from the linear fit of the depuration curve delivered the best precision, the data from this method were used for the assessment. In addition, this method resulted in the highest BCF. BCF_k = 23804 L/kg - BCF_L: normalised to 5% lipid content (mean lipid content during plateau phase: 6.97%; value from report: 6.5%) o C_{fish_L} = 0.05/0.0697*572 mg/kg = 410 mg/kg o BCF_{ss_L} = 410 mg/kg / 27.5 µg/L = 14910 L/kg o BCF_{k_L} = 0.05/0.0697*23804 L/kg = 17076 L/kg **Upper treatment level** - $BCF_{ss} = C_{fish\_ss}/C_w$ = 3769 mg/kg / 263.4 µg/L = 14309 L/kg (recalculated; BCF_{ss} value from report: 15060 L/kg) - BCF_k: The kinetic constants were determined according to three methods. For results see Table A7_4_2_1-7. Since k1, k2 and BCF derived from the linear fit of the depuration curve delivered the best precision, the data from this method were used for the assessment. In addition, this method resulted in the highest BCF. BCF_k = 16738 L/kg - BCF_L: normalised to 5% lipid content (mean lipid content during plateau phase: 6.77%; value from report: 6.5%) o C_{fish_L} = 0.05/0.0677*3769 mg/kg = 2784 mg/kg o BCF_{ss_L} = 2784 mg/kg / 263.4 µg/L = 10569L/kg o BCF_{k_L} = 0.05/0.0677*16783 L/kg = 12395 L/kg	x x x
4.1.5	Uptake and depuration rate constants	See Table A7_4_2_1-7.	
4.1.6	Depuration time	No data given on DT50 or DT90	
4.1.7	Metabolites	Not applicable	
4.1.8	Other Observations	If the addition of the test item to the upper treatment surpassed 90% of the water solubility, or if the stock solution was not directed into the outflow of the pump, undissolved particles of the test item could be observed. No details are given on the occasions when this happened.	
4.2	**Estimation of bioconcentration**	The BCF was estimated to be 488 using the BCFBAF module v 3.01 of EPI Suite v4.10 (US EPA, 2012). M-DCPP is within the estimation domain for non-ionic substances of the BCF module within the BCFBAF v3.01 model. The training set for the BCF module included 466	

compounds. The molecular weight (269.13 g/mol) and log Kow (4.58) each fall within the range of the training set for non-ionic compounds (i.e., the MW range of the training set is 68.08 g/mol to 959.17 g/mol; the log Kow range of the training set is -1.37 to 11.59).

The biotransformation half-life was estimated to be 11.3 d normalised to 10 g fish. The training set included for the biotransformation model 421 compounds. The range of the molecular weight is 68.08 to 959.17 g/mol (average: 259.75 g/mol). The range of the log Kow values is 0.31 to 8.70. The substance falls within the estimation domain of the models with a molecular weight of 269.13 g/mol and a log Kow of 4.58.

The fragments of the substance are covered by the characteristics of the substances of the training sets regarding identity and maximum number of occurrence. Therefore, it can be concluded that the substance is within the estimation domain of the biotransformation model of BCFBAF v3.01.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	With one exception the validity criteria can be considered to be fulfilled (see Table A7_4_2_1-8). During the uptake period the test concentration in the lower treatment level, the test concentration was not maintained within the limit of ±20% of the mean measured values. Nevertheless since this happened only once during the relevant uptake period from day 0 to day 19, this can be considered as having only a slight effect on the bioconcentration kinetics. Significant details are missing in the documentation of the study (see 5.3.2 Deficiencies). Data are missing on the concentration of the solvent. A solvent control was not performed. Part of the data were re-evaluated since the original data assessment was not reproducible.	x
5.2	**Results and discussion**	<u>Experimental studies</u> - BCF (lower treatment // upper treatment): o BCFss = 20800 // 14309	x
		o BCF_{ss_L} = 14910 // 10569	
		o BCF_k = 23804 // 16738	
		o BCF_{k_L} = 17076 // 12395	x
		o The max. BCF derived from this study is 23804 ($logBCF_k$ = 4.4). - Uptake rate constant (lower treatment // upper treatment): k1 = 3592 // 2437 - Depuration rate constant (lower treatment // upper treatment): k2 = 0.1509 // 0.1456	
5.3	**Conclusion**	The study has significant deficiencies in the performance. Therefore the results should be considered with caution for the assessment of the bioaccumulation potential	

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

		The experimentally determined BCFs for M-DCPP range from 10569 to 23804 (log BCF = 4.0 to 4.4). These values are above the threshold values for very bioaccumulative substances (> 5000 L/kg).	x
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes	x

- No information on solvent (methanol) concentration in stock/test solutions (max. level according to OECD TG 305: 100 mg/L or 0.1 mL/L; same level in all concentration levels) given.
  No solvent control was run.
  No mortality was observed during exposure and depuration period; therefore the used concentration was below the toxic level. Behavioural abnormalities were only observed in the highest treatment level, which are most likely due to the test item itself (see following paragraph). It can be concluded that the solvent did not cause toxic effects.
- In the upper treatment level, the fish did not take up any food during the uptake phase.
  The animals showed normal behaviour during the depuration period, indicating that the effect was reversible and was probably caused by the test item. In the highest treatment level the fish lost weight, while in the control and the lower treatment level no loss of weight was detectable. The lipid content declined in all fish over the uptake period. The weight loss might have affected the uptake of the test item or the concentration in the fish. In the first case, the fish might have had accumulated more if their feeding behaviour had not been affected. In the second case, the test item might show higher concentrations due to a lower fish weight compared to the amount of test item taken up by the fish.
- Data do not indicate if calculation basis is wet weight or dry weight. Wet weight is assumed for this assessment based on a missing description of dry matter determination and/or dry matter content of fish.
- The test concentration in the lower treatment was not maintained within ±20% of the mean concentration. On two occasions, the concentration was 33% lower than the mean value (19 mg/L compared to 28 mg/L; days 3 and 20).
- Lower treatment level: Two measured values of the test item concentration in fish were marked as outliers (Table A7_4_2_1-6) and described as not being considered in the further assessment. A determination of the test item concentration in the other replicate was not performed. Nevertheless, the data were used for the determination of the mean concentration and the BCF as well as the kinetic constants k1 and k2. In addition if the last two data points which were marked as outliers are taken out of the assessment, the plateau phase was not reached yet.
- Initial fish weight (506 ± 38 mg) stated in report does not reflect the size of the fish sampled during the study. Fish were bigger with just one exception. See Tables A7_4_2_1-4 and A7_4_2_1-5.

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

- Detection/quantification limit of analytical method not specified.
- Calibrated sector of method given as 5 to 50 mg/L. Compared to the concentration range of the study (approx. 30 to 300 µg/L), this range seems inadequate for a precise analytical determination.

Evaluation by Competent Authorities

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	August 2013
Materials and Methods	3.3.1: Table references should be included. 3.3.1: Exchange in table A7_4_2_1-1 Preparation of TS solution for poorly or volatile test substances: "No data given" by "The test item was added in methanol: for the upper treatment vessel: 10g/L for the lower treatment vessel: 1g/L" 3.3.1: Exchange in table A7_4_2_1-2 Dilution water in section pH: "No data" by "8.28; pH following CaCO3 saturation 8.13"
Results and discussion	4.1.2: The table numeration needs adaptation: Second Table A7_4_2_1-6 should be Table A7_4_2_1-7 4.1.3: Exchange "3769" by "3823" and "541" by "527". 4.1.3: Exchange "4499" by "4489". 4.1.4: Table A7_4_2_1-7 should be A7_4_2_1-8 4.1.4: Exchange "BCF_L: normalised to 5% lipid content (mean lipid content during plateau phase: 6.97%; value from report: 6.5%) o C_{fish_L} = 0.05/0.0697*572 mg/kg = 410 mg/kg o BCF_{ss_L} = 410 mg/kg / 27.5 µg/L = 14910 L/kg o BCF_{k_L} = 0.05/0.0697*23804 L/kg = 17076 L/kg by "BCF_L: normalised to 5% lipid content (mean lipid content 6.8% excluding one outlier: 12.52 % from day 1 as this value is quiet far from usually reported lipid contents for *Danio rerio;* value from report: 6.5%) o C_{fish_L} = 0.05/0.068*572 mg/kg = 420 mg/kg o BCF_{ss_L} = 420 mg/kg / 27.5 µg/L = 15273 L/kg o BCF_{k_L} = 0.05/0.068*23804 L/kg = 17503 L/kg 4.1.4: Exchange "BCF_L: normalised to 5% lipid content (mean lipid content during plateau phase: 6.77%; value from report: 6.5%) o C_{fish_L} = 0.05/0.0677*3769 mg/kg = 2784 mg/kg o BCF_{ss_L} = 2784 mg/kg / 263.4 µg/L = 10569L/kg o BCF_{k_L} = 0.05/0.0677*16783 L/kg = 12395 L/kg by"BCF_L: normalised to 5% lipid content (mean lipid content 6.9% as stated in the report) o C_{fish_L} = 0.05/0.069*3823 mg/kg = 2770 mg/kg o BCF_{ss_L} = 2770 mg/kg / 263.4 µg/L = 10517 L/kg o BCF_{k_L} = 0.05/0.069*16738 L/kg = 12129 L/kg

Section A7.4.2 Bioconcentration in aquatic organisms

Annex Point IIA7.5

Conclusion	5.1: Table A7_4_2_1-8 should be A7_4_2_1-9 5.2: Exchange o BCFss = 20800 // 14309 o BCF_{ss_L} = 14910 // 10569 by o BCFss = 20800 // 14514 o BCF_{ss_L} = 15273 // 10517 5.2: Exchange o BCF_{k_L} = 17076 // 12395 by o BCF_{k_L} = 17503 // 12129 5.3: Exchange “10569” by “10517”. 5.3.2: Addition: Several parameter are not measured/stated in the report: total solids, TOC (only DOC is stated), alkalinity. Although both sexes were used no documentation of non-significance was provided. No information on age of the test animals or detailed information on feeding composition is provided. Fish weight data are not linked to individual fish chemical concentrations. No statement regarding growth of the fish is made.
Reliability	2
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Particularly the BCF values gained in the lower treatment regime can be considered valid: No signs of toxicity were observed. During the uptake phase was not maintained within the limit of ±20% of the mean measured values. Nevertheless since this happened only once during the relevant uptake period from day 0 to day 19 this can be considered to have only a slight effect on the bioconcentration kinetics. The solvent concentration in the test vessel is not given. Nevertheless, a rough estimation of the methanol content based on a concentration of 1g Methyl-DCPP/L methanol and an obtained concentration of about 30 µg Methyl-DCPP/L water in the test vessel results in an assumed methanol concentration of 0.03 ml/L or 0.024 mg/L. According to the test guideline this solvent concentration can be considered to have an insignificant effect on the maximum dissolved concentration of the test substance. The average lipid content of 6.8% for the lower treatment is obtained excluding one outlier (12.52 % on day 1 as this value is quiet far from usually reported lipid contents for *Danio rerio*). This value is nearly the same as for the average lipid content of the control (6.7%) and the upper treatment (6.9%). As growth was of minor importance in the depuration period growth dilution does not seem to be of importance.

Table A7_4_2_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Vehicle	Solvent used: Methanol
Concentration of vehicle	No data given
Vehicle control performed	No
Other procedures	-

Table A7_4_2_1-2: Dilution water

Criteria	Details
Source	Chlorine-free drinking water
Alkalinity	No data
Hardness	Uptake period: 0.90 - 1.05 mmol/L as $CaCO_3$ Depuration period: 0.93 - 0.96 mmol/L as $CaCO_3$
pH	No data
Oxygen content	No data
Conductance	246 µS/cm
Holding water different from dilution water	No

Table A7_4_2_1-3: Test organisms

Criteria	Details
Species/strain	Zebra fish (D*anio rerio*)
Source	[redacted]
Wild caught	No
Age/size	Mean wet weight: 506 ± 38 mg Mean length: 3.1 ± 0.3 cm
Kind of food	Warm-water fish food: - tablets: Pet's Best Tabs - flakes: Zierfisch Futter - food analysed for methyl-diclosan: no traces detected
Amount of food	1-2% of body weight per day
Feeding frequency	3 times per day
Pretreatment	ACCLIMATION - Acclimation period: 14 d - Acclimation conditions (same as test or not): yes - Type and amount of food: same as above - Feeding frequency: same as above - Health during acclimation (any mortality observed): mortality < 5% during housing period of 14 d prior to test start
Feeding of animals during test	See above

Table A7_4_2_1-4: Test system

Criteria	Details
Test type	Flow-through regime
Renewal of test solution	5 volumes per day (preheated water: 210 mL/min; stock solution: 6.18 µL/min)
Volume of test vessels	Glass aquaria, 40*60*40 cm³, nominal volume: 80 L, fill volume: 60 L
Volume/animal	1 L/animal
Number of animals/vessel	60
Number of vessels/ concentration	One aquarium
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_2_1-5: Test conditions

Criteria	Details
Test temperature	23±2 °C
Dissolved oxygen	Uptake period: 6.8 - 8.6 mg/L Depuration period: 8.5 - 9.6 mg/L
pH	Uptake period: 7.7 - 8.2 Depuration period: 8.1 - 8.3
Conductance	Uptake period: 248 - 259 µS/cm Depuration period: 248 - 259 µS/cm
Total organic carbon	Depuration period: < 1 ppm (on one occasion: 1.48 ppm in lower treatment and 1.35 ppm in upper treatment)
Adjustment of pH	No
Aeration of dilution water	Yes
Intensity of irradiation	No data (use of fluorescent tubes)
Photoperiod	12 hours light and 12 hours dark

Table A7_4_2_1-6: Lipid content, fish weight and measured concentrations in test medium and fish during uptake period (outliers according to report marked with *)

Day of exposure	M-DCPP					Lipid content (%)			Fish weight (g)		
	water (µg/L)	% of mean	in fish (mg/kg)								
			Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean
Control											
0.33	Not applicable					9.28		9.28	0.8585		0.8585
1						9.10		9.10	0.7509		0.7509
3						8.51		8.51	0.8049		0.8049
6						6.82		6.82	0.7308		0.7308
10						7.82		7.82	0.7679		0.7679
14						7.69		7.69	0.8589		0.8589
17						6.28		6.28	0.8644		0.8644
18						6.13		6.13	1.1136		1.1136
19						6.38		6.38	1.0245		1.0245
20											
Mean						**7.56**			**0.86**		
Lower treatment											
0	32	17%									
0.33	32	17%	77		77	8.44		8.44	0.7372		0.7372
1	33	20%	201		201	12.52		12.52	0.8386		0.8386
3	19	-31%	247		247	9.88		9.88	1.4924		1.4924
6	26	-5%	356		356	6.42		6.42	0.9080		0.9080
10	26	-5%	558		558	9.33		9.33	0.8317		0.8317
14	25	-9%	626		626	7.82		7.82	0.8672		0.8672
17	29	6%	736		736	5.86		5.86	0.7142		0.7142
18	30	9%	409*			6.76		6.76	1.0860		1.0860
19	31	13%	533*			5.42	6.60	6.01	0.9646	0.8295	0.8971
20	19	-31%									
Mean	**27.5**		**plateau phase: 572**			**8.12**			**0.93**		
Upper treatment											
0	244	-7%	355		355	8.08		8.08	1.1020		1.1020

Day of exposure	M-DCPP					Lipid content (%)			Fish weight (g)		
	water (µg/L)	% of mean	in fish (mg/kg)								
			Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean
0.33	296	12%									
1	267	1%	879		879	8.28		8.28	0.8514		0.8514
3			1981		1981	8.71		8.71	0.8200		0.8200
6	270	2%	2800		2800	7.60		7.60	0.7521		0.7521
10	314	19%	3094		3094	7.27		7.27	0.6123		0.6123
14	223	-15%	3835	4347	4091	6.84	8.75	7.80	0.5303	0.6841	0.6072
17			2372*	3696	3696	5.00	7.17	6.09	0.4895	0.7133	0.6014
18	245	-7%	3475		3475	6.40		6.40	0.5977		0.5977
19	262	-1%	4489	2485*	4489	7.87	4.74	6.31	0.5595	0.6238	0.5917
20	250	-5%									
Mean	**263.4**		**plateau phase: 3769**			**7.39**			**0.73**		

Table A7_4_2_1-6: Lipid content, fish weight and measured concentrations in fish during depuration period (outliers according to report marked with *)

Day of exposure	M-DCPP in fish (mg/kg)			Lipid content (%)			Fish weight (g)		
	Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean	Repl. 1	Repl. 2	Mean
Control									
0.17	Not applicable			4.15		4.15	0.8416		0.8416
0.33				6.05		6.05	0.8497		0.8497
1				6.09		6.09	0.9212		0.9212
3				6.24		6.24	0.8518		0.8518
6				6.08		6.08	0.8308		0.8308
9				6.19		6.19	0.9382		0.9382
13				6.22		6.22	1.0408		1.0408
18				4.79		4.79	0.8728		0.8728
Mean						**5.73**			**0.8934**
Lower treatment									
0.17	130			4.69		4.69	1.0444		1.0444
0.33	96			5.54		5.54	1.1967		1.1967
1	257	65*	257	7.18	5.47	6.33	0.8773	0.9277	0.9025
3	158		158	8.99		8.99	0.9902		0.9902
6	75		75	5.67		5.67	0.8204		0.8204
9	54		54	5.73		5.73	0.769		0.7690
13	65		65	7.58		7.58	0.9273		0.9273
18	18		18	5.33		5.33	0.5171		0.5171
Mean						**6.23**			**0.8960**
Upper treatment									
0.17	2756		2756	7.02		7.02	0.8416		0.8416
0.33	3601		3601	7.24		7.24	0.8497		0.8497
1	3674	3278	3476	5.55	9.26	7.41	0.9212		0.9212
3	2050		2050	6.68		6.68	0.8518		0.8518
6	593	4347	2470	4.63		4.63	0.8308		0.8308
9	829	3696	2263	6.6		6.60	0.9382		0.9382
13	788		788	4.72		4.72	1.0408		1.0408
18	224		224	7.38		7.38	0.8728		0.8728
Mean						**6.46**			**0.8934**

Table A7_4_2_1-7: BCF and kinetic constants determined with three different methods for lower and upper treatment level

Parameter	Value	Error	RSD (%)
Lower treatment level			
Determined from uptake period			
k1	4690	1105	23.56
k2	0.2142	0.06	28.80
BCF	21895		52.37
Determined from depuration period			
k1	8079	2026	25.08
k2	0.4325	0.08	18.64
BCF	18680		43.71
Determined from linear fit of depuration curve			
k1	3592	87	5.20
k2	0.1509	0.02	11.46
BCF	**23804**		16.66
Upper treatment level			
Determined from uptake period			
k1	3113	375.8	12.07
k2	0.202	0.0302	14.95

Parameter	Value	Error	RSD (%)
BCF	15411		27.02
Determined from depuration period			
k1	2628	435	16.55
k2	0.1826	0.0279	15.28
BCF	14392		31.83
Determined from linear fit of depuration curve			
k1	2437	64	2.63
k2	0.1456	0.0136	9.34
BCF	**16738**		11.97

Table A7_4_2_1-8: Validity criteria for bioaccumulation test according to OECD Guideline 305 (1996)

	fulfilled	Not fulfilled
The water temperature variation is less than ± 2°C	**X**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**X**	
Concentration of the test item in the chambers is maintained within ± 20% of the mean of the measured values during the uptake phase	**(X)**	**(X)**
Concentration of the test substance is below its limit of solubility in water, taking into account the effect that the test water may have on effective solubility	**X**	
Mortality of control animals <10%	**X**	

Section 7.4.3.4 Annex Point IIIA XIII.2.4 Effects on reproduction and growth rate with an invertebrate species

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1999): Influence of [redacted] on survival and reproduction of *Daphnia magna* in a semistatic test over three weeks. [redacted] Project No. [redacted] 1999-11-02 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD Guideline No. 211 (1998)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	[redacted] (DCPP)	
3.1.1	Lot/Batch number	Batch No. [redacted]	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	> 99%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	Stability in water: Hydrolytically stable (determined in RCC Project 712260)	
		Solubility in water: 19.5 mg/L, sd ±0.3 mg/L (determined in RCC Project 712012)	x
3.1.6	Method of analysis	HPLC-UV/VIS-detection	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test media were prepared as follows: at the start of each treatment period a stock solution of nominal 5 mg/L was freshly prepared by mixing 5.0 mg of the test item homogeneously in 1.0 litre test water by ultrasonification and intensive stirring. For this the following series of operations was used: 5.0 g test item ad 100 mL test water - ultrasonic treatment for 15 minutes and shaking - addition of 600 mL test water - ultrasonic treatment for 15 minutes - addition of 300 mL test water - ultrasonic treatment for 15 minutes - stirring for 3 hours at room temperature in the dark. After this treatment most of the test item was dissolved in the stock solution. A small amount of test item was homogeneously suspended. In this way the use of an organic solvent could be avoided at the dosage of the test item into the test media.	
		Then, adequate volumes of the intensively stirred stock solution were	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII.2.4

		mixed into test water to obtain the desired test concentrations. The test media were always freshly prepared before introduction of the test animals. See table A7_4_3_4-1.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water	See table A7_4_3_4-2	
3.4.2	Test organisms	See table A7_4_3_4-3	x
3.4.3	Handling of offspring	According to the test guideline	x
3.4.4	Test system	See table A7_4_3_4-4	
3.4.5	Test conditions	See table A7_4_3_4-5	x
3.4.6	Duration of the test	21 days	
3.4.7	Test parameter	The mortality, the number of young born and signs of intoxication were compared with corresponding parameters in the control.	
3.4.8	Examination / Sampling	The mortality of adults and the number of young were recorded at least three times per week before renewal of test media. Dead animals and offspring were removed at the renewal of the test media. The reproduction rate was calculated as the total number of living offspring produced per parent female alive at the end of the test. The pH-values and dissolved oxygen concentrations in the test media were measured in all test concentrations and in the control at the beginning and end of each treatment period. Only the pH-values and oxygen concentrations of the lowest and highest test concentration and the control are reported since in all test media pH-values and oxygen concentrations were in the same range. The water temperature was measured in one control beaker at the start of the test and at the end of each treatment period. Additionally, the air-temperature in the testing room was continuously recorded. The appearance of the test media was recorded at each of the above mentioned intervals.	x
3.4.9	Monitoring of TS concentration	For the analysis of the test item concentrations, single samples were taken from the freshly prepared stock solution and duplicate samples from the freshly prepared test media (without food and daphnia) of all test concentrations and the control at the first treatment period (Day 0), at a treatment period in the second week (Day 12) and at a treatment period in the last week (Day 16). For the determination of the maintenance of the test item concentrations during the test medium renewal periods, the following additional samples were taken at the end of two treatment periods (on Days 14 and 19):	x

Section 7.4.3.4 Annex Point IIIA XIII.2.4 Effects on reproduction and growth rate with an invertebrate species

		a) from sufficient volumes of the freshly prepared test media of all test concentrations and the control which were incubated during the renewal periods under the same conditions as the test itself, however without food particles and test animals. b) from the test media of all test concentrations and the control out of the actual test (including the food particles) by pouring together the contents of the test beakers after treatment period. The first of these two stability control treatments lasted for 48 hours, the second for 72 hours (weekend), corresponding to the two different renewal periods of test media. The concentrations of the test item FAT 80‘2201A were analyzed in all duplicate test media samples from the test concentrations of nominal 0.22 and 0.46 mg/L. From the control only one of the samples was analyzed from each of all sampling dates.
3.4.10	Statistics	The NOEC and the LOEC of the reproduction rate were statistically evaluated by testing the mean reproduction rate at the test concentrations on statistically significant differences to the control value by the multiple Williams-test (Ref. 1, 2) after a one-way analysis of variance (ANOVA). Additionally, the EC_{50} of the reproduction rate after 21 days was statistically evaluated by Probit Analysis. The 95% confidence limits could not be calculated.
		4 RESULTS
4.1	**Range finding test**	Acute test RCC project 712203 was taken into consideration (see Study Summary A7_4_1_2)
4.1.1	Concentrations	
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Initial concentrations of test substance	The test concentrations were based on the results of an acute toxicity test with *Daphnia magna* (RCC project 712203) and on the results of a pre-experiment to the solubility of the test item. The initial concentrations of the test article were: 0.022, 0.046, 0.10, 0.22, and 0.46 mg/L, and a control.

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII.2.4

4.2.2	Actual concentrations of test substance	In the analyzed freshly prepared test media the mean measured test item concentrations varied in the range of 73 to 103% of the nominal values.	x, x
		In the samples without food particles the mean test item concentrations at the end of the renewal periods of 48 and 72 hours were in the range of 83 - 91% of the nominal values. The total mean measured concentrations, calculated as the average over all measurements per test concentration (samples without food only) were 81 and 82%, respectively. Under the test conditions without food and Daphnia, DCPP was sufficiently stable. Therefore, all reported biological results are related to the nominal concentrations of the test item.	x, x x
		In the samples including food particles the mean test item concentrations at the end of the renewal periods of 48 and 72 hours decreased to 41 - 63% of the nominal values. Thus, a part of the test item had obviously adsorbed onto the food particles. However, since filter feeding test organisms as daphnids may take up the test item also from ingested food, the decrease of the test item concentrations in the test media due to adsorption onto food is not taken into account.	x
4.2.3	Effect data	Mortality: In the control and the test concentrations up to and including 0.22 mg/L the survival rate of the test animals at the end of the test was 100%. At the next higher concentration (the highest test concentration of 0.46 mg/L), all test animals had successively died until Day 21. Thus, the survival rate of *Daphnia magna* after 21 days was significantly (>20%) reduced first at the test concentration of 0.46 mg/L.	x
		Reproduction rate: The first young offspring released from their parent animals were recorded in the control and at the test concentrations up to 0.22 mg/L on Day 9 of observation. At the highest concentration tested (0.46 mg/L) no offspring were reproduced. In the control one test animal was obviously sterile, since it did not reproduce till the end of the test. This test animal was not taken into account at the calculation of the reproduction rate.	x
		The mean reproduction rate of the daphnids in the control was 98 ± 11 alive offspring per adult (mean ± SD). According to the results of a Williams-Test (one-sided smaller, $\alpha = 0.05$) no significant toxic effect of the test item on the mean reproduction rate was determined up to and including the test concentration of 0.22 mg/L. At the next higher test concentration of 0.46 mg/L, the mean reproduction rate of surviving daphnids could not be calculated, since none of the parent animals survived until the end of the test.	
		The 21-day EC_{50} for the reproduction rate of *Daphnia magna* was calculated to be 0.30 mg/L (95% confidence limits could not be calculated).	
		Results are summarised in tables A7_4_3_4-6, A7_4_3_4-7 and A7_4_3_4-8.	
4.2.4	Concentration / response curve	Not applicable, since the NOEC and LOEC were very close from each other at 0.22 and 0.46 mg/L respectively, and no concentrations were tested between those.	x
4.2.5	Other effects	No	
4.3	**Results of controls**	See tables A7_4_3_4-6, A7_4_3_4-7 and A7_4_3_4-8	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII.2.4

4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of the test item FAT 80'220/A on the reproduction and survival rate of *Daphnia magna* was investigated in a semistatic test over 21 days following the OECD Guidelines for Testing of Chemicals No. 211. The nominal test concentrations were 0.022, 0.046, 0.10, 0.22, and 0.46 mg/L, and a control.	
5.2	**Results and discussion**	Taking into account the survival rates and the reproduction rates of the test animals, the highest concentration of DCPP tested without toxic effects after the exposure period of 21 days (21 -day NOEC) was 0.22 mg/L. The lowest concentration tested with toxic effects (21-day LOEC) was determined to be 0.46 mg/L due to the 100% mortality rate of *Daphnia magna* at this test concentration. The total mean measured concentrations (calculated as the average over all relevant measurements per test concentration) in the analyzed test media of 0.22 and 0.46 mg/L nominal were 81 and 82%, respectively. Under the test conditions (without food and Daphnia), DCPP was sufficiently stable. Therefore, all reported biological results are related to the nominal concentrations of the test item.	x x x
5.2.1	NOEC (21 d)	0.22 mg/L	x
5.2.2	LOEC (21 d)	0.46 mg/L (at the LOEC 100% mortality was observed)	x
5.2.3	EC_{50}	0.30 mg/L	x
5.3	**Conclusion**	The validity criteria were fulfilled and are summarised in table A7_4_3_4-9.	x
5.3.1	Reliability	1	
5.3.2	Deficiencies	-	

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

January 2011

Materials and Methods

3.1.5: *Addition*: "Vapour pressure: 1.2 x 10^{-06} Pa at 25 °C (determined in RCC Project 711990)

3.4.2: Regarding Table A7_4_3_4-3: On day 9/16/19-20 the food amount provided was 0.25 mg TOC/test animal/day thereby exceeding the recommended range of 0.1 to 0.2 mg C/Daphnia/day

3.4.3: *Replacement*: Replace "According to the test guideline" by "Dead animals and offspring were removed at the renewal of the test media."

3.4.5: Regarding Table A7_4_3_4-5: The intensity of the light is given in lux.

3.4.8: Dead animals and offspring were not removed daily as recommended by Test Guideline No.211, but only at the renewal of the test media.

3.4.9: The test concentrations were not measured in appropriate intervals: No data are available regarding test concentration at the time of renewal in the first week. Moreover, due to the potential adsorption properties it could not be expected that the test concentration remains in the $\pm$ 20% of the nominal. Hence, all test concentration, when freshly prepared and at renewal should have been measured.

Results and discussion

4.2.2: Addition: Add "Actual test concentrations are reported for the two nominal test item concentrations of 0.22 and 0.46." before term "In"

4.2.2: Addition: Add "aged" before term "samples"

4.2.2: Add "and without test animals" after term "particles".

4.2.2: Deletion: Delete "Under the test conditions without food and Daphnia, DCPP was sufficiently stable. Therefore, all reported biological results are related to the nominal concentrations of the test item."

4.2.2: *Replacement*: Substitute "However, since filter feeding test organisms as daphnids may take up the test item also from ingested food, the decrease of the test item concentrations in the test media due to adsorption onto food is not taken into account."

by

"Most likely, additional test material has entered daphnia via uptaken food. Nevertheless, the amount of test substance adsorbed to removed offspring as well as the amount of test substance adsorbed to food consumed by the offspring, which was not removed daily but only at the renewals, is unclear. Therefore, the averaged test item concentrations from day 14 and 19, for which the used test media per test concentration were poured together after removal of daphnia and which had included food particles, are considered the more reliable test item concentrations, reflecting actual test conditions.

The averaged measured test item concentrations from day 14 and 19 including food particles are 0.094 mg/L for the nominal concentration of 0.22 mg/L, and 0.27 mg/L for the nominal test item concentration of 0.46 mg/L."

4.2.3: *Addition*: Add "based on nominal test item concentrations" after term "Mortality"

	4.2.3: *Addition*: Add “based on nominal test item concentrations” after term “Reproduction rate”.
	4.2.4: *Addition*: Add “(based on nominal test item concentrations)” after term “0.46 mg/L”)
Conclusion	5.2: *Addition* Add “As the amount of test substance adsorbed to removed offspring as well as the amount of test substance adsorbed to food consumed by the offspring, which was not removed daily but only at the renewals, is unclear, the averaged test item concentrations from day 14 and 19, for which the used test media per test concentration were poured together after removal of daphnia and which had included food particles, are considered the more reliable test item concentrations, reflecting actual test conditions.” before term “Taking”.
	5.2: *Replacement*: Substitute “0.22mg/L” by “0.094 mg/L (nominal concentration of 0.22 mg/L)”
	5.2: *Replacement*: Substitute “0.46mg/L” by “0.27 mg/L (nominal concentration of 0.46 mg/L)”
	5.2: Deletion: Delete “The total mean measured concentrations (calculated as the average over all relevant measurements per test concentration) in the analyzed test media of 0.22 and 0.46 mg/L nominal were 81 and 82%, respectively. Under the test conditions (without food and Daphnia), DCPP was sufficiently stable. Therefore, all reported biological results are related to the nominal concentrations of the test item.”
	5.2.1: *Replacement*: Substitute “0.22mg/L” by “0.094 mg/L (nominal concentration of 0.22 mg/L)”
	5.2.2: *Replacement*: Substitute “0.46mg/L” by “0.27 mg/L (nominal concentration of 0.46 mg/L)”
	5.2.3: *Addition:* Add based on nominal concentrations after term “mg/L”
	5.3: Correction in title of table A7_4-3_4-9: Exchange “6” by “9”
	5.3.1: Change to “2”
	5.3.2: Addition: Additionally to the statements given above, no raw data on temperature measurements are provided and the hardness was not measured after start of the test.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_4_3_4-1: Preparation of TS Solution for Poorly Soluble or Volatile Test Substances

Criteria	Details
Dispersion	Yes
Vehicle	No
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_4_3_4-2: Dilution Water

Criteria	Details
Source	The test was conducted in reconstituted water (purified water with analytical grade salts and additives), “M7“.
Salinity	-
Hardness	2.5 mmol/L (=250 mg/L) as $CaCO_3$
pH	Initial: 7.9 ± 0.3
Ca / Mg ratio	Not stated
Na / K ratio	Not stated
Oxygen content	Before use, the test water was aerated until oxygen saturation
Conductance	No data
TOC	No data
Holding water different from dilution water	Not specified

Table A7_4_3_4-3: Test Organisms

Criteria	Details
Strain / Clone	Females of a clone of the species *Daphnia magna* Straus.
Source	The clone was originally supplied by the [redacted] in 1992, defined from the supplier as clone 5. Since this date the clone is bred in the laboratories of [redacted].
Age	< 24 hours
Breeding method	In the laboratories of [redacted] the test animals were bred under identical temperature and light conditions as in the test, and in the same kind of test water as used in the test.
Kind of food	The test animals were fed with a food mixture containing one part of green algae of the species *Scenedesmus subspicatus* (freshly grown in the laboratories of RCC) and one part of fish food suspension* *10 g of a commercial fish diet TETRA MIN Hauptfutter were powdered and suspended in 500 mL test water. The suspension was allowed to stand for 4 hours, then 400 mL of the supernatant were taken and boiled. This suspension was stored deep frozen in small quantities until use.
Amount of food	The amounts of TOC fed per test animal and day: Day 0/1: 0.10 mg TOC/test animal/day Day 2/5-8/12-13: 0.15 mg TOC/test animal/day Day 14-15: 0.20 mg TOC/test animal/day Day 9/16/19-20: 0.25 mg TOC/test animal/day The carbon content of the food suspensions was determined using a Shimadzu TOC 500 Analyser. The food amounts were based on the measured concentration of total organic carbon (TOC) in the food suspensions.
Feeding frequency	The test animals were fed on each working day (Monday - Friday).
Pre-treatment	No
Feeding of animals during test	Yes, see above

Table A7_4_3_4-4: Test System

Criteria	Details
Test type	Semistatic conditions
Renewal of test solution	The test media of all test concentrations and of the control were renewed on Days 2, 5, 7, 9, 12, 14, 16 and 19 of the exposure period (every Monday, Wednesday and Friday). By that, a total of 9 treatments were performed. At these dates the surviving test animals were carefully transferred by glass tubes from the old test vessels into the freshly prepared test media of the corresponding concentrations.
Volume of test vessels	Each test animal was kept individually in a 100 mL glass beaker containing 80 mL test medium. The beakers were covered with glass plates.
Volume/animal	80 mL/ 1 parent daphnids in the beginning.
Number of animals/vessel	1 animal/vessel
Number of vessels/concentration	10 vessels (replicates) per concentration and a control
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test Conditions

Criteria	Details
Test temperature	20 - 21 °C during the test period
Dissolved oxygen	>8.2 mg O_2/L during the test
pH	7.6 to 8.0 during the test
Adjustment of pH	No
Aeration of dilution water	Yes: before use, the basis solution was aerated and not during the test
Quality/Intensity of irradiation	300 - 800 Lux
Photoperiod	16 hour/day

Table A7_4_3_4-6: Number of surviving test animals, exposed to the test item

Exposure Day	Nominal concentration of the test item (mg/L)					
	Control	0.022	0.046	0.10	0.22	0.46
0	10	10	10	10	10	10
1	10	10	10	10	10	10
2	10	10	10	10	10	7
5	10	10	10	10	10	3
7	10	10	10	10	10	2
9	10	10	10	10	10	2
12	10	10	10	10	10	1
14	10	10	10	10	10	1
16	10	10	10	10	10	1
19	10	10	10	10	10	1
21	10	10	10	10	10	0
% surviving on Day21	100*	100	100	100	100	0

* All test animals survived, however one of the test animals was obviously steril and was therefore not taken into account at the calculation of the reproduction rate.

Table A7_4_3_4-7: The total number of alive, young daphnids reproduced by all adults (cumulative values).

Exposure Day	Nominal concentration of the test item (g/L)					
	Control	0.022	0.046	0.10	0.22	0.46
0	0	0	0	0	0	0
1	0	0	0	0	0	0
2	0	0	0	0	0	0
5	0	0	0	0	0	0
7	0	0	0	0	0	0
9	110	114	115	104	91	0
12	300	319	308	304	302	0
14	489	641	518	617	491	0
16	594	644	618	646	653	0
19	725	779	748	797	845	0
21	883	847	908	919	968	0
% of control[1]	100	95.9	102.8	104.1	109.6	0

[1] based on the value of the last exposure day

Table A7_4_3_4-8: The number of alive offspring reproduced per surviving adult within 21 days of exposure.

Replicate No.	Nominal concentration of the test item *(mg/L)*					
	Control	0.022	0.046	0.10	0.22	0.46
1	113	89	100	99	109	*
2	92	73	88	89	89	*
3	78	91	89	98	88	*
4	99	70	74	81	109	*
5	*	89	101	84	91	*
6	89	75	104	74	90	*
7	104	84	96	98	95	*
8	106	83	86	77	96	*
9	93	85	91	108	93	*
10	109	108	79	111	108	*
mean	98.1	84.7	90.8	91.9	96.8	-
±*SD*	11.1	10.9	9.7	12.8	8.6	-
n	9*	10	10	10	10	-
CV%	11.3	12.9	10.7	14.0	8.8	-
Mean in %	100.0	86.3	92.5	93.7	98.7	-
STAT	-	*n.s.*	n.s.	n.s.	n.s.	-

CV %: coefficient of variation in %: ($SD/mean_x$) x 100%
STAT: results of a Williams-test with the mean values of alive offspring (one-sided smaller, $\alpha = 0.05$)
n.s.: mean value not significantly lower than in the control
* This test animal was obviously steril and was therefore not taken into account at the calculation of the reproduction rate

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	Fulfilled	**Not fullfilled**
Mortality of parent animals < 20% at test termination	**X**	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	**X**	

Criteria for poorly soluble test substances	**X**	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species

Annex Point IIIA XIII 2.4

Official use only

1 REFERENCE

1.1	**Reference**	[redacted](2003). Determination of the effect on reproduction of *Daphnia magna* after exposition to [redacted] Report no. [redacted] Date: 2001-04-01 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes - OECD 211 (1998)
2.2	**GLP**	Yes
2.3	**Deviations**	Yes - On day 20, only 0.125 mg C were fed to the *Daphnia*, since no more algal solution was in stock. Since the test was finished the day after this deviation is considered to have had no influence on the outcome of the test. - Filtration of samples for chemical analyses filtered with membrane filters up to day 13 according to study plan; from day 14 paper filters were used deviating from the study plan. No significant difference was recognized between the results from both methods.

3 METHOD

3.1	**Test material**	[redacted](Methyldiclosan, degradation product of Diclosan)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	Deviating from specification given in section 2 as follows
3.1.3	Purity	99.7%
3.1.4	Composition of Product	Not applicable
3.1.5	Further relevant properties	-appearance: white powder -water solubility: approx. 0.4 mg/L -homogeneous and stable, not volatile
3.1.6	Method of analysis	Gas chromatography (GC) -solvent: iso-octane -internal standard: hexychlorbenzene

Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

		-gas chromatograph: GC hp 6890 A, Agilent Technologies -column: Phen_ex 7HG-G002-11, 30 m, 0.25 mm, 0.25 µm -GC method: 02080201G -linear sector of method: 10-50 mg/L -R of calibration function: 0.9997 -standard deviation of injection: ± 3.6% -recovery rate (water) at 90%: 77±6% -validation report [redacted]	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	A super-saturated stock solution of nominal 100 mg/L was prepared for the nominal test concentration of 100 mg/L. See also table A7_4_3_4-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	Not applicable	
3.4	**Testing procedure**		
3.4.1	Dilution water	M4 medium (see table A7_4_3_4-2)	x
3.4.2	Test organisms	*Daphnia magna* (see table A7_4_3_4-3)	
3.4.3	Handling of offspring	Vessels inspected for offspring daily on weekdays and once per weekend. Offspring transferred to larger beakers (2/concentration), which were inspected daily for dead animals.	
3.4.4	Test system	Semi-static (see table A7_4_3_4-4)	
3.4.5	Test conditions	Give relevant test conditions in tabular form (see table A7_4_3_4-5)	x, x, x
3.4.6	Duration of the test		
3.4.7	Test parameter	Reproduction: fecundity (number of young per female and day) Mortality of parental animals	
3.4.8	Examination / Sampling	Give details on examination/sampling intervals and procedure	
3.4.9	Monitoring of TS concentration	Yes See table A7_4_3_4-6 for intervals	x
3.4.10	Statistics	Time-weighted average was applied according to the guideline (OECD 211).	
		4 RESULTS	
4.1	**Range finding test**	Performed (GLP-guideline study according to OECD 202: 48-h EC50 > 0.15 mg a.s./L, mean measured)	
4.1.1	Concentrations	Limit test (super-saturated test solution at 100 mg/L)	

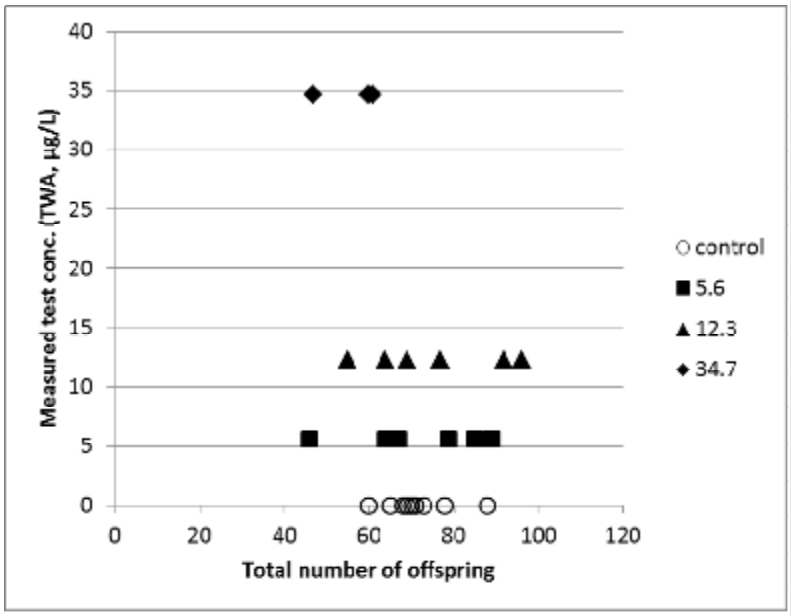
Section 7.4.3.4 Annex Point IIIA XIII 2.4 — Effects on reproduction and growth rate with an invertebrate species

4.1.2	Number/ percentage of animals showing adverse effects	1 animal (5%) was immobile after 48 h.	
4.1.3	Nature of adverse effects	Immobility	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Geometric series of diluted super-saturated stock solution (100%, 50%, 25%, 12.5%, 6.25%; see Table A7_4_3_4-6)	
4.2.2	Actual concentrations of test substance	See Table A7_4_3_4-6	
4.2.3	Effect data	See Table A7_4_3_4-7 and A7_4_3_4-8.	x

	Effect values			
	Report (nominal, %saturation)	**Report (measured, mean initial)**	**Recalculated (TWA)**	x
	Reproduction (µg a.s./L)			x
NOEC	12.5%	36.8/34.7*	12.3	x
LOEC	25%	79.3	34.7	x
EC50	> 25%	> 79.3	> 34.7	x
	Parental mortality (µg a.s./L)			x, x
NOEC	< 6.25**	not reported	< 5.6	x
LOEC	6.25**		5.6	x
EC50	12.5 – 25**		approx. 20.7	x

*: Deviating values were given in report.

**: Effect values were not given in report.

4.2.4	Concentration / response curve		
4.2.5	Other effects	Not reported	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

4.3	**Results of controls**		
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	Not applicable	
4.4.2	Results	Not applicable	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The chronic toxicity of M-DCPP was studied in reproduction test using *Daphnia magna* (Ciba, 2003; A[redacted]. The study was performed under GLP according to OECD 211. The test solutions were renewed three times per week (semi-static exposure). The test concentrations were analytically monitored (new and aged test solutions).	
5.2	**Results and discussion**	The test concentrations caused a high mortality of the parental animals. Three out of 10 animals died already in the lowest test solution. Any offspring from these animals was excluded from the statistical evaluation. As described in the previous acute toxicity studies, the test item recovery in the solutions was low. Since the result was based on mean initial test item concentrations, the effect values were re-calculated using a time-weighted average (TWA). The 21-d NOEC has been recalculated yielding a value of 0.012 mg/L (measured, TWA). The parental mortality of this test solution was 40%.	x
5.2.1	NOEC	Reproduction : 12.3 µg a.s/L	x
5.2.2	LOEC	Reproduction : 34.7 µg a.s/L	x
5.2.3	EC_{50} (EC_x)	Reproduction : > 34.7 µg a.s/L	x
5.3	**Conclusion**	The validity criteria for the control replicates were kept. Therefore the study is to be regarded as valid according to OECD 211. Nevertheless, the poor analytical recovery of the test item in the aged test solutions is critical. In addition the high parental mortality at the selected test concentrations should be regarded as a deficiency of this study. According to OECD 211 “the range of test concentrations should preferably not included any concentrations that have a statistically significant effect on adult survival since this would change the nature of the test […].”	
5.3.1	Reliability	3	
5.3.2	Deficiencies	Yes - Poor analytical recovery of the test item in the aged test solutions - High parental mortality at the selected test concentrations; according to OECD 211 “the range of test concentrations should preferably not include any concentrations that have a statistically significant effect on adult survival since this would change the nature of the test […].” - The effect concentrations are based on the initially measured	

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

concentrations of the new test solutions. Since the fate of the test item is not proven and effects were not only observed directly after addition of the test solutions, the effect values should be based on average concentrations (preferably time-weighted average). This is a methodological error.
The time-weighted average concentrations have been recalculated for this robust study summary.

Section 7.4.3.4 Effects on reproduction and growth rate with an invertebrate species
Annex Point IIIA XIII 2.4

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	September 2013
Materials and Methods	3.4.1 in table A7_4_3_4-2: *Addition in box for hardness*: "hardness in test solution: 260-305 mg $CaCO_3$/L".
	3.4.5 in table A7_4_3_4-5: box for test temperature: Exchange "20.7" with "21.4".
	3.4.5 in table A7_4_3_4-5: box for pH: Exchange "6.7" by "7.2".
	3.4.5 in table A7_4_3_4-5: box for photoperiod: Exchange "e.g. 12 h photoperiod daily" by "No data for the actual test but 16/8 given in the Holding and Breeding section".
	3.4.9 in table A7_4_3_4-6: box for TWA-values: Exchange "5.6" by "4.9", "12.3" by "11.6", "34.7" by "33.8" and "56.1" by "54.9".
Results and discussion	4.2.2: for actual TWA values in table A7_4_3_4-6 see amendement in 3.4.9
	4.2.3: Exchange term "Reproduction" by "Production of living offspring per surviving parent organism".
	4.2.3: Exchange "12.3" by "11.6".
	4.2.3: Exchange "34.7" by "33.8".
	4.2.3: Exchange "34.7" by "33.8".
	4.2.3: Exchange "Parental mortality" by "Reproductive output per parent animal in the start of the test which did not inadvertently or accidently die during test"
	4.2.3: Exchange "5.6" by "4.9".
	4.2.3: Exchange "5.6" by "4.9".
	4.2.3: Exchange "approximately 20.7" by ">11.6"
Conclusion	5.2: Exchange "0.012 mg/L (measured, TWA). The parental mortality of this test solution was 40%." by "0.0049 mg/L (mean measured, based on Reproductive output per parent animal in the start of the test which did not inadvertently or accidently die during test"
	5.2.1: Exchange "12.3" by "<4.9".
	5.2.2: Exchange "12.3" by "<4.9".
	5.2.3: Exchange ">34.7" by ">11.6".
Reliability	2
Acceptability	Acceptable for environmental risk assessment.
Remarks	At the lowest measured test concentration of 4.9 µg/L (TWA) an effect was observed leading to an unbound NOEC.

Table A7_4_3_4-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	No solvent used
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	Filtration (0.45 µm)

Table A7_4_3_4-2: Dilution water

Criteria	Details
Source	M4-medium (Drinking water with an enrichment of minerals is used in the test (calcium chloride, magnesium sulphate, sodium chloride, potassium chloride).)
Salinity	No data
Hardness	Not specified
pH	Drinking water: 8.48
Ca / Mg ratio	Not specified
Na / K ratio	Not specified
Oxygen content	No data
Conductance	262 µS/cm
TOC	
Holding water different from dilution water	No

Table A7_4_3_4-3: Test organisms

Criteria	Details
Strain / Clone	*Daphnia magna* (STRAUS), strain: Berlin
Source	[redacted]
Age	0 to 24 h
Breeding method	Females multiplied by parthenogenesis Holding: glass beakers (2 L), medium M4 (Elendt), feeding with, change of medium twice a week, photo period 16/8 h (fluorescent tubes), T = 21±2°C
Kind of food	Tetra Aminin® (cold-water fish food) suspended in M4 and boiled for three seconds; green algae *Pseudokirchneriella subcapitata*
Amount of food	No data
Feeding frequency	No data
Pretreatment	30-min settling-in period (animals which showed no apparent damage were used in the test)
Feeding of animals during test	Yes Unicellular green alga (*Pseudokirchneriella subcapitata*) 0.15 mg org. C/animal/day

Table A7_4_3_4-4: Test system

Criteria	Details
Test type	Semi-static
Renewal of test solution	Three times per week
Test vessels	Glass beakers
Volume of test vessels	100 mL (nominal), 80 mL fill volume
Volume/animal	80 mL/parent animal
Number of animals/vessel	1
Number of vessels/ concentration	10
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_4-5: Test conditions

Criteria	Details
Test temperature	- 19.7 to 20.7 °C (test concentrations with living daphnids at test end) - 19.8 to 21.8 °C (test concentrations where all parental animals did not survive until test end)
Dissolved oxygen	8.6 to 9.8 mg/L
pH	Control: 6.5 to 8.0 Test concentrations: new: 6.0 to 7.3; aged: 6.7 to 8.4
Adjustment of pH	No
Aeration of dilution water	Dilution water was aerated prior to preparation of test solutions. Test solutions were not aerated.
Quality/Intensity of irradiation	≤ 2000 Lux
Photoperiod	e.g. 12 h photoperiod daily

Table A7_4_3_4-6: Analytically determined test concentrations (TWA = time-weighted average)

Saturation (%)	Solution	Measured test item concentraion (µg/L) on day									Mean (µg/L)	TWA (µg/L)
		0	2	5	7	9	12	14	16	21		
0	new	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0
	aged	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.1	
6.25	new	7.5	24.2	18.6	15.0	20.7	7.8	23.7	20.2	-	17.2	5.6
	aged	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	7.7	1.0	
12.5	new	16.5	49.6	42.2	37.5	36.4	23.5	41.4	47.1	-	36.8	12.3
	aged	-	5.4	0.0	2.9	0.4	0.0	0.6	0.0	11.0	2.5	
25	new	74.7	105.9	91.3	73.4	69.4	53.1	92.1	74.3	-	79.3	34.7
	aged	-	21.9	4.9	44.8	6.3	0.0	0.0	0.0	42.2	15.0	
50	new	158.0	188.3	178.2	145.1	140.2	-	-	-	-	162.0	56.1
	aged	-	31.8	6.7	30.8	19.9	0.0	-	-	-	17.8	
100	new	345.6	410.1	-	-	-	-	-	-	-	377.9	181.4
	aged	-	90.6	53.8	-	-	-	-	-	-	72.2	

Table A7_4_3_4-7: Mortality of parent animals (M) and number of offspring

(In the treatment 50%, all parent animals except for replicate 7 and 8 were found dead on day 5. In the treatment 100% all parent animals were found dead on day 4).

conc. in %	day													
	8	9	11	12	13	14	15	16	17	18	19	20	21	Σ
0	7	0	14	0	0	26	0	24	0	0	17	0	0	88
	0	0	9	0	0	23	0	0	18	0	0	18	0	68
	0	0	8	0	0	22	0	0	20	0	0	20	0	70
	0	0	12	0	21	0	0	20	0	0	0	18	0	71
	0	0	10	0	19	0	0	0	20	0	0	20	0	69
	0	0	9	16	0	0	0	20	0	0	20	0	0	65
	0	0	11	0	0	20	0	0	23	0	0	24	0	78
	M													
	0	0	8	0	18	0	0	15	0	0	0	19	0	60
	0	0	11	0	24	0	0	0	18	0	0	20	0	73
6,25	0	8	M											
	0	5	0	16	0	0	3	19	0	0	21	0	0	64
	0	8	0	16	0	0	21	0	0	21	0	0	0	66
	0	9	0	16	0	0	0	21	0	0	21	0	0	67
	0	10	0	18	0	0	29	0	0	22	0	0	0	79
	0	8	0	14	0	0	25	0	0	22	0	0	20	89
	0	9	M											
	0	8	M											
	0	10	0	15	18	0	0	24	0	0	18	0	0	85
	0	5	0	0	0	0	0	20	0	0	21	0	0	46
12,5	0	11	0	17	0	0	25	0	1	23	0	0	0	77
	0	12	M											
	0	6	0	17	0	0	12	0	0	16	4	0	0	55
	0	0	M											
	0	4	0	19	0	0	0	20	0	0	21	0	0	64
	0	0	10	0	18	0	0	22	0	0	19	0	0	69
	10	0	15	0	0	23	0	0	12	10	0	0	26	96
	7	0	16	0	0	26	0	0	0	20	0	0	23	92
	0	13	M											
	0	0	M											
25	0	6	0	0	17	0	0	23	0	0	7	7	0	60
	0	4	0	0	12	0	0	17	0	0	14	0	0	47
	0	0	M											
	0	0	M											
	0	0	M											
	0	0	M											
	0	4	4	9	0	0	25	0	0	18	0	0	0	60
	0	0	M											
	0	0	9	0	0	16	0	16	0	0	0	0	20	61
	0	0	M											
50														
	0	0	0	M										
	0	0	0	M										

Table A7_4_3_4-8: Fecundity (living offspring per parent animal alive at test termination)

Saturation%	control (0)	6.25	12.5	25	50	100
Parent animals alive at test end	9	7	6	4	0	0
Mortality	10%	30%	40%	60%	100%	100%
Mean	71	71	76	57	0	0
Min	60	46	55	47	0	0
Max	88	89	96	61	0	0
SD	8	15	16	7	-	-
CV	0.11	0.21	0.21	0.12	-	-
Fecundity (% of control)	-	99%	106%	80%	0%	0%

Table A7_4_3_4-6: Validity criteria for invertebrate reproduction test according to OECD Guideline 211

	fulfilled	Not fullfilled
Mortality of parent animals < 20% at test termination	X	
Mean number of live offspring produced per parent animal surviving at test termination ≥ 60	X	

Section A7.4.3.5.1 Annex Point IIIA, XIII.3.4	Effects on sediment dwelling organisms	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	A test with sediment dwelling organisms is not required for biocidal active substances used as disinfectant (Biocidal Product Types 1, 2 and 4, see TNsG for Data Requirements (April 2000), chapter 2.5). However, a study with *Chironomus riparius* exposed to the structurally similar active substance triclosan (CAS-No. 3380-34-5) is available. The phenol ring of triclosan and DCPP is identical. Furthermore, since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that triclosan has a higher potential for bioaccumulation and a higher toxicity to organisms than DCPP. Thus, it is considered justified to bridge the results of the study performed with triclosan to DCPP.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	June 2011	
Evaluation of applicant's justification	Agree with applicant's version.	
Conclusion	Agree with applicant's version.	
Remarks	-	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted](2006): Triclosan: Effects on the development of sediment-dwelling larvae of *Chironomus riparius* in a water-sediment system with spiked sediment. [redacted] Report No. [redacted] date: 2006-07-17 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 14 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	OECD Guidelines for Testing of Chemicals, Guideline 218: Sediment-Water Chironomid Toxicity Test Using Spiked Sediment (adopted 13 April 2004).
2.2	**GLP**	Yes
2.3	**Deviations**	None
		3 MATERIALS AND METHODS
3.1	**Test material**	**[^{14}C]-Triclosan** (5-Chloro-2-(2,4-dichloro-[U-^{14}C]phenoxy)-phenol) diluted in the unlabelled test item.
3.1.1	Lot/Batch number	labelled TS: [redacted] unlabelled TS: [redacted]
3.1.2	Specification	labelled TS: Specific radioactivity: 5.43 MBq/mg non-labelled TS: -
3.1.3	Purity	labelled TS: 87.3%, was purified before use to 95% non-labelled TS: 99.6%
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	Solubility in water: 12 mg/L (data as given in the report); 0.012 g/L at 20 °C (Pointurier, 1990; cf. section 2 of III-A)
3.1.6	Method of analysis	The radioactivity in the water, pore water and sediment samples was measured by means of liquid scintillation counting (LSC). The concentration of the parent molecule and its metabolites were additionally analysed by HPLC in the water and pore water samples as well as sediment extracts from all samples taken on Day 0, 7 and at the test termination (Day 28) from the test concentrations of nominal 25 and 100 mg/kg.
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	Due to the low solubility of Triclosan in water the application solutions were prepared in acetone with concentrations of 0.56, 1.10, 2.22 4.43 and 8.86 mg/mL and then applied to a portion of sand before spiking the sediment. For spiking the sediment, volumes of 1.0 to 1.25 mL of the

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

		corresponding application solution were applied to 10 g sand in a glass flask. The solvent acetone was completely evaporated by a stream of nitrogen at room temperature under a hood over night. Thereafter, 120 g of the wet sediment was weighed to the 10 g sand/test item mixture in the glass flask. To improve the mixing procedure of the spiked sediment, 10 mL of the test water were added. This spiked sediment was intensively shaken by hand and then mixed in the closed glass flasks on a roller mixer for about 2 hours. Afterwards the sediment was filled into the test beakers. The sediment of the solvent control was prepared like the spiked sediments incorporating the same amount of acetone to the sand, but without the test item. To the sediment of the control 10 g of sand was added accordingly, but without acetone or test item.	
3.3	**Reference substance**	For co-chromatography unlabelled Triclosan and methyl-Triclosan were used.	x
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Dilution water, Test sediment	For moistening purified water was used. For details on dilution water see table A7_4_3_5_1-1. The test sediment used was artificial sediment which was prepared blending all the constituents, adding sufficient water. The pH was adjusted following different steps to a final pH of 7.2. This wet sediment was filled into the test beakers. For further details on test sediment see table A7_4_3_5_1-1a	
3.4.2	Test organisms	*Chironomus riparius*, see table A7_4_3_5_1-2	
3.4.3	Test system	The spiked wet artificial sediment was filled into each test vessel at a layer of approximately 2 cm depth. This amount corresponded to 130 g wet weight with 46% water content (or 89 g dry sediment). Then, 240 mL of test water were poured into each beaker very slowly, taking care not to disturb the sediment. This water volume corresponded to a water column of 5.5 cm depth. Thus, the ratio of the depth of the sediment layer to the depth of the overlying water was 1:2.8 (and did not exceed 1:4). The vessels were prepared two days before inserting of the test animals, and were incubated under the test conditions to reach equilibrium between sediment and aqueous phases (pore water and overlying water) in the water-sediment systems. See table A7_4_3_5_1-3 for more details.	
3.4.4	Test conditions	See table A7_4_3_5_1-4	
3.4.5	Duration of the test	28 days	
3.4.6	Test parameter	The number of emerged adults and their sex. Any other signs of intoxication of the larvae, pupae and emerged midges were recorded. Water hardness, concentration of ammonium, water temperature, pH and concentration of dissolved oxygen were measured to check the water quality.	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

3.4.7	Sampling	The number of emerged adults and their sex was recorded daily from Day 10 after application until Day 28 (7 days after emergence of the last test animals in the controls). After sex identification the midges were removed from the vessels and discarded. Water temperature, pH and concentration of dissolved oxygen were measured in all test vessels during the incubation time of the water-sediment systems (before the insertion of the larvae). During the study period these parameters were measured once per week and at study termination. The dissolved oxygen concentrations were additionally measured in the test media of all four test beakers per treatment two additional times a week. The water temperature was additionally measured in the test media of one of the four test beakers per treatment two times a week. The air temperature in the temperature controlled testing room was continuously recorded as well. Water hardness and concentration of ammonium were measured in one test beaker of the control, the solvent control and the highest test concentration at the start and end of the test.	
3.4.8	Monitoring of TS concentration	Water, pore water and sediment samples from the test systems were taken on Day 0 (insertion of the larvae), on Day 7 and at the test termination on Day 28. The test systems of the middle and highest test concentration (25 and 100 mg/kg) and the solvent control were sampled at the start and end of the test. On Day 28 analytical samples were taken out of one test beaker from the biological test. The analytical samples from Day 0 and 7 were taken from test beakers prepared exclusively for analytical measurements in parallel to the biological test (two test beakers were prepared for each sampled test concentration, and one for the solvent control). One of the two treated test beakers and the solvent control beaker were sampled on Day 0. The other treated replicates were incubated under the conditions of the test until sampling on Day 7. To these additional replicates test organisms were inserted and food was added as in the biological part of the study.	x
3.4.9	Statistics	The **emergence ratio** and the development time and rate were calculated for each test vessel. The emergence ratio is defined as the sum of fully emerged midges divided by the number of inserted larvae. The mean **development time** represents the mean time span between the insertion of the larvae (Day 0) and the emergence of the experimental cohort of midges. The **development rate** is the reciprocal of the development time (unit: 1/day) and represents that portion of larval development which takes place per day. The development rate is preferred for the evaluation of these sediment toxicity studies as its variance is lower, more homogeneous and it is closer to normal distribution as compared to development time. Hence, the development rate is used instead of development time. For both, emergence ratio and development rate the arithmetic mean value (mean), standard deviation (SD), minimum and maximum (min/max) were calculated from the four replicates per treatment. The mean emergence ratios and development rates of all test concentrations were statistically evaluated on significant differences to the solvent control by the multivariate Dunnett-test after a one-way analysis of variance (ANOVA). Statistical evaluations were done separately for	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

		emerged males and females (development rate) and with pooled sexes (emergence ratio). The mean emergence ratio and development rate in the control were compared to the solvent control by STUDENT t-tests.	
		4 RESULTS	
4.1	**Limit Test**	Not performed	
4.1.1	Concentration	-	
4.1.2	Number/ percentage of animals showing adverse effects	-	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentrations: 6.3, 12.5, 25, 50 and 100 mg/kg dry sediment plus non-treatment control group and a solvent control group with the auxiliary solvent acetone. The test concentrations were based on the results of a previous range-finding toxicity test.	
4.2.2	Actual concentrations of test substance	The analytically measured concentrations of Triclosan in the sediment samples ranged from 92 to 97% of the nominal values. The concentrations of Triclosan in the sediment were constant over the test period of 28 days. The concentrations of Triclosan respectively the radioactive residues in the overlaying water columns were very low throughout the test period (<1% of applied radioactivity). Also in the pore water samples very low amounts of radioactivity were found (0.1% of applied radioactivity). Up to five different metabolites were detected, but no individual metabolite accounted for more than 4% of the applied radioactivity (Ml, M2, M3 and M5, M15). All reported biological results are related to the nominal concentrations of the test item in the sediment since the analytical measurements confirmed the correct preparation of the sediments.	x x
4.2.3	Effect data	For the number of emerged midges see table A7_4_3_5_1-5. Development rate is shown in table A7_4_3_5_1-6.	
4.2.4	Concentration / response curve	Dose-effect-curves on number of emerged midges and on development rate are not given because these are not applicable (no effect at the highest concentration tested)	
4.2.5	Other effects	No symptoms of toxicity were observed at the larvae, pupae and emerged midges during the study.	
4.3	**Results of controls**	The emergence ratios per vessel in the controls ranged from 85 to 100%. The midges in the control and the solvent control had emerged between Day 12 and 21 (and thus fulfilled the validity criterion of the test guideline requesting the emergence between Day 12 and 23). The mean emergence and development rates of the midges in the solvent control	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

		was not significantly different to the control.	
4.4	**Test with reference substance**	Not performed	
4.4.1	Concentrations	-	
4.4.2	Results	-	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Toxic effects of the test item Triclosan on the development of sediment-dwelling larvae of the midge *Chironomus riparius* in a water-sediment system was investigated following the OECD guidelines 218 using spiked sediment (adopted 13 April 2004). The radiolabelled test item was applied to the sediment in a static water-sediment system. The nominal initial test item concentrations in the sediments were 6.3, 12.5, 25, 50 and 100 mg Triclosan per kg dry sediment. A control (water-sediment systems without test item application) and a solvent control group (acetone) were tested in parallel. The spiked sediments were kept for two days under the test conditions to reach equilibrium between sediment and aqueous phases as pore water and overlying water. Then, first-instar larvae of *Chironomus riparius* were exposed for a period of 28 days until full maturation of the larvae to adult midges. The test parameters of the study were development time/rate of the midges and emergence ratio as the number of fully emerged male and female midges.	
5.2	**Results and discussion**	The analytically measured concentrations of Triclosan in the sediment samples ranged from 92 to 97% of the nominal values. The concentrations of Triclosan in the sediment were constant over the test period of 28 days. The concentrations of Triclosan respectively the radioactive residues in the overlaying water columns were very low throughout the test period (<1% of applied radioactivity). Also in the pore water samples very low amounts of radioactivity was found (0.1% of applied radioactivity). This means that Triclosan was mainly bound to the sediment, but most of this fraction was extractable. Up to five different metabolites were detected, but no individual metabolite accounted for more than 4% of the applied radioactivity. All reported biological results are related to the nominal concentrations of the test item in the sediments since the analytical measurements confirmed the correct preparation of the sediments. Up to and including the highest test concentration of 100 mg/kg the mean emergence ratios and the mean development rates of the midges were not significantly lower than in the solvent control. Thus, the 28-day **NOEC of Triclosan was at least 100 mg/kg dry weight sediment**. The NOEC might even be higher, but concentrations in excess of 100 mg/kg have not been tested. The 28-day LOEC was clearly higher than 100 mg/kg dry sediment. This value could not be quantified due to the absence of toxicity of Triclosan at the test concentration of 100 mg/kg.	x x
5.3	**Conclusion**	No effects were observed concerning the emergence ratio and the development rate after 28 days. Therefore the highest concentration	

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

		tested was considered the NOEC.		
		The concentrations of Triclosan in the sediment were constant over the test period of 28 days The concentrations of Triclosan respectively the radioactive residues in the overlaying water columns were very low throughout the test period (<1% of applied radioactivity). Also in the pore water samples very low amounts of radioactivity was found (0.1% of applied radioactivity). This means that Triclosan was mainly bound to the sediment, but most of this fraction was extractable.	x x	
		The test showed no major deviations from the Guideline (see table A7_4_3_5_1-7) and the results can be regarded as valid.		
5.3.1	Reliability	1		
5.3.2	Deficiencies	-		x

Section A7.4.3.5.1 Effects on sediment dwelling organisms

Annex Point IIIA, XIII.3.4 *Chironomus riparius*

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	May 2011
Materials and Methods	3.3: Replace “and methyl-Triclosan were used.” by “was used and as a reference item methyl-Triclosan was used to characterise the radioactive degradation products formed.”
	3.4.4: Table A7_4_3_5_1-4: Change > 5.7 mg/L into ≥ 5.7 mg/L in the box for Dissolved oxygen
	3.4.7: *Addition* after “additional times a week”: “in the first three weeks and one additional time in the fourth week.
	3.4.7: *Addition* after “two times a week”: “for the first three weeks and one additional time in the fourth week”.
	3.4.8: Replace “Water, pore water and sediment samples from the test systems were taken on Day 0 (insertion of the larvae), on Day 7 and at the test termination on Day 28. The test systems of the middle and highest test concentration (25 and 100 mg/kg) and the solvent control were sampled at the start and end of the test.” by “Water, pore water and sediment samples were taken from the test systems of the middle and highest test concentration (25 and 100 mg/kg) on Day 0 (insertion of the larvae), on Day 7 and at the test termination on Day 28. The solvent control was sampled at the start and end of the test.”
Results and discussion	4.2.2: Change “<1%” to “≤1%”.
	4.2.2: Change “0.1%” to “≤0.1%”.
	5.2: Change “<1%” to “≤1%”.
	5.2: Change “0.1%” to “≤0.1%”.
Conclusion	5.3: Change “<1%” to “≤1%”.
	5.3: Change “0.1%” to “≤0.1%”.
	5.3.2: *Addition*: One test beaker of the nominal test item concentration of 50 mg/kg was lost by accident on day 11 of the study.
	5.3.2: *Addition*: According to OECD test guideline 218 the ratio of the depth of the sediment layer to the depth of the overlying water should be 1:4.
Reliability	1, as only minor deviations occurred to OECD test guideline 218 and the study was performed according to GLP.
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	The test concentrations are based on a range finding study, but no effects are observed at the highest tested concentration. The results from the range finding study should preferably have been included.

Table A7_4_3_5_1-1: Dilution water

Criteria	Details
Source	The test and breeding water used were reconstituted water ("M7-medium").The medium is prepared using purified (deionised) water and adding analytical grade salts and additives. The concentrations of them in the water are given in chapter 2.4.4 of the report.
Alkalinity	Not specified
Hardness	Initial: 2.0 mmol/L (= 200 mg/L) as $CaCO_3$ During the test: 2.9 to 3.8 mmol/L
pH	Initial: 7.9 ± 0.3
Ca / Mg ratio	Ca^{2+} + Mg^{2-} -ions: approx. 2.5 mmol/L
Na / K ratio	Not specified
Oxygen content	At least 5.7 mg/L
Conductance	No data
Holding water different from dilution water	No

Table A7_4_3_5_1-1a: Test sediment

Sediment characterisation	Details
Particle size distribution	The artificial sediment was prepared on the basis of dry weights as follows: Sphagnumpeat: 5% (air dried, very finely ground to 1 mm, organic carbon content 43.3%) Kaolin clay: 20% (content of A1203: 36.4%) Sand* (Sihelco 36): 75% Size distribution: 0.25 mm 1.0% 0.18 mm 8.0% 0.125 mm 80.0% 0.09 mm 10.0% <0.09 mm 1.0% Calcium carbonate ($CaCO_3$): 0.23% * The amount of sand was reduced in this mixture according to the amount of sand added by the application of the test item (10 g/test beaker)
Organic carbon (%)	-
Water content (%)	46% (based on dry weight) of the final mixture
pH	Initial: 7.2 The pH of the peat suspension was 5.5 at start of moistening. $CaCO_3$ was added for pH adjustment, and after the moistening procedure the pH was 6.1. After blending of all the constituents and adding water to the 46% moisture, a sediment sample of this final mixture, of about 10 g was shaken with 25 mL of a 0.1 mol KCl solution for 30 minutes. The pH of the final mixture of sediment was 7.2.
TOC (Total organic carbon)	2.2% (TOC based on dry weight)

Table A7_4_3_5_1-2: Test organisms

Criteria	Details
Strain	*Chironomus riparius*, first larval stage
Source	In-house culture [redacted].)
Age (at start of the study)	At the date when the test animals were placed into the test beakers, the larvae were 2-3 days old
Breeding method	Only fresh egg masses were used as source for the test animals. Fresh eggs were bred under similar temperature and light conditions as in the test, and in the same kind of test water as used in the test.
Kind of food	Tetra Min® fish food (supplied by TETRA-Werke, D-49304 Melle, Germany) was used as food for the larvae during the test.
Amount of food	The following amounts of dry Tetra Min® were fed per vessel and day: On Days -2/0/1/4: 0.467 mL/vessel 23 mg Tetra Min®/vessel On Days 6/8/11/13/15/18/20/23/26: 0.934 mL/vessel 47 mg Tetra Min®/vessel
Feeding frequency	The fish food flakes were finely ground and suspended in test water. Food was added at least three times per week until Day 26, when all of the larvae had emerged. The first portion of food was added after the preparation of the water-sediment systems and one other portion before introduction of the larvae to allow ingestion of contaminated food already at the start of the test.
Pretreatment	Five and six days before inserting the larvae into the test beakers some fresh egg masses were taken from the test organism culture and deposited into small vessels in test water with a small amount of food (mixture of fresh green algae *Desmodesmus subspicatus* from a laboratory culture and a Tetra Min® fish food suspension).
Feeding of animals during test	Yes

Table A7_4_3_5_1-3: Test system

Criteria	Details
Static test	Each beaker contained approx. 2 cm high layer of sediment (130 g ww sediment); approx. 0.24 L water (overlying water). The ratio of the depth of the sediment layer to the depth of the overlying water was 1:2.8 (and did not exceed 1:4).
Volume of test vessels	Glass beakers (600 mL, approximately 8 cm in diameter) were used as test vessels. The beakers were covered with watch-glasses. From Day 8 until the end of the test each test beaker was additionally covered with a mosquito net to prevent the escape of emerged adult test animals.
Volume water/animal	12 mL/animal (240 mL/20 animals)
Number of animals/vessel	20
Number of vessels/ concentration	4 replicates (test beakers) per test concentration, the control and solvent control. Additional replicates were prepared in parallel for the analytical requirements.
Test performed in closed vessels due to significant volatility of TS	No

Table A7_4_3_5_1-4: Test conditions

Criteria	Details
Test temperature	Water temperature: 19.9 - 21.5°C during the experiment. The study was performed in a temperature-controlled room. The water temperature differed less than ±1.000 between beakers at any time during the test.
Dissolved oxygen	> 5.7 mg/L (= 65% oxygen saturation value)
pH	During the test period: pH 7.4 to 8.5
Adjustment of pH	Yes
Aeration of dilution water	Gentle aeration in water during exposure (except 24 h after addition of larvae)
Quality/Intensity of irradiation	Light intensity: approx. 500-700 Lux
Photoperiod	16:8 light-dark-cycle with a 30 minute transition period between light and darkness.

Table A7_4_3_5_1-5: Emergence ratio (male and female midges pooled)

	Nominal initial test item concentration (mg/kg)						
	Control	Solvent control	6.3	12.5	25	50	100
Sum of inserted larvae per treatment	80	80	80	80	80	60*	80
Sum of emerges midges per treatment	73	73	77	75	73	56	73
% of emerged midges per treatment (mean)	91	91	96	94	91	93	91
Emergence ratio ERarc: mean	1.310	1.273	1.471	1.354	1.273	1.313	1.278
SD	0.1772	0.0482	0.1988	0.15181	0.0482	0.0556	0.0835
Min	1.173	1.249	1.173	1.249	1.249	1.249	1.173
Max	1.571	1.345	1.571	1.571	1.345	1.345	1.345
n	4	4	4	4	4	3	4
% of solvent control	103	100	116	106	100	103	100
STAT	n.s.*	---	n.s.	n.s.	n.s.	n.s.	n.s.

ER_{arc}: arcsin-transformed emergence ratio
STAT: results of a Dunnett-test (cL = 0.05, one-sided smaller)
n.s.: mean ERarc not significantly lower than in the solvent control
n.s*.: mean ERarc not significantly different from the solvent control (based on a t-test, c. = 0.05, two-sided)
s.: mean ERarc significantly lower than in the solvent control

* One test beaker was lost by accident on Day 11 of the study. This replicate is therefore excluded from the evaluation of the results.

Table A7_4_3_5_1-6: Development rate over 28 days for males and females

Development rate per treatment (day-1)	Males						
	control	solvent control	6.3	12.5	25	50	100
Mean	0.0762	0.0746	0.0736	0.0758	0.0740	0.0746	0.0772
SD	0.00182	0.00206	0.00504	0.00169	0.00454	0.00132	0.00133
min	0.0747	0.0719	0.0674	0.0740	0.0683	0.0734	0.0753
max	0.0788	0.0769	0.0788	0.0779	0.0789	0.0760	0.0783
n	4	4	4	4	4	3	4
%of solvent control	102	100	99	102	99	102	104
STAT	n.s.			n.s.	n.s.	n.s.	n.s.

Development rate per treatment (day1)	Females						
	control	solvent control	6.3	12.5	25	50	100
Mean	0.0656	0.0673	0.0652	0.0676	0.0642	0.0661	0.0644
SD	0.00147	0.00302	0.00165	0.00189	0.00102	0.00072	0.00270
min	0.0637	0.0636	0.0631	0.0663	0.0634	0.0653	0.0612
max	0.0671	0.0710	0.0671	0.0704	0.0655	0.0666	0.0678
n	4	4	4	4	4	3	4
% of solvent control	98	100	97	100	95	98	96
STAT	n.s.*	---	n.s.	n.s.	n.s.	n.s.	n.s.

Table A7_4_3_5_1-7: Validity criteria for sediment-water chironomid toxicity test using spiked sediment according to OECD Guideline 218

	Fulfilled	Not fulfilled
Mean emergence in the control ≥ 70%	**X**	
Emergence of adults in the control between day 12 and 23 after start of exposure	**X**	
Dissolved oxygen concentration in at least one replicate per concentration and control ≥ 60 % of the air saturation value	**X**	
pH of the overlying water at the end of the test between 6 - 9	**X**	
Water temperature difference not more than ± 1°C	**X**	

Section A7.5.1.1 **Annex Point IIA VII.7.4**	**Inhibition to microbiological activity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	Since DCPP containing biocidal products are used indoor, only sewage treatment plants will be the receiving compartment for DCPP residues. However, sewage sludge might be applied to soil as fertilizer. Therefore, soil can be contaminated indirectly. Since the compound is inherent biodegraded in the sewage treatment plant, residues of DCPP in the sewage sludge are low and thus, only low amounts of the active substance might reach the soil compartment. Studies on respiration and nitrification in soil conducted with the structurally similar active substance triclosan (CAS-No. 3380-34-5) are available. The phenol ring of triclosan and DCPP is identical. Furthermore, since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that triclosan has a higher toxicity to organisms than DCPP. Thus, it is considered justified to bridge the results of the study performed with triclosan to DCPP.	x x
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	June 2011	
Evaluation of applicant's justification	Exchange "Since DCPP containing biocidal products are used indoor, only sewage treatment plants will be the receiving compartment for DCPP residues." by "According to the Intended Use (Doc. II-B) no direct exposure to surface water, only indirect exposure via STP is possible." Exchange "inherent biodegraded" by "eliminated to a high degree".	
Conclusion	Agree with applicant's version with amendments given above.	
Remarks	-	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (02)

Annex Point IIA7.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2007a): The Effects of Triclosan on Soil Respiration. [redacted] Report No. [redacted] date: 2007-02-21 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes; OECD Guideline 217 (2000)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	**Triclosan**	
3.1.1	Lot/Batch number	[redacted]	
3.1.2	Specification	-	
3.1.3	Purity	99.3 %	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	The CO_2 evolved was measured with an infrared (IR) gas analyser. The CO_2 evolved from each sample was calculated based on the accumulation time, the flow rate, the measurement period, and the concentration of carbon dioxide in the outlet air stream.	
3.2	**Reference substance**	Yes, dinoseb acetate	
3.2.1	Method of analysis for reference substance	Not analysed, only CO_2	
3.3	**Testing procedure**		
3.3.1	Soil sample / inoculum / test organism	See table A7_5_1_1-1.	
3.3.2	Test system	Soil samples of approximately 150 g dry weight were set-up in 1-litre incubation flasks closed with cotton wool plugs. Control samples were not treated with the test item. Treatment V samples had a concentration of 2.0 mg Triclosan/kg dry soil. Samples of	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (02)

Annex Point IIA7.4

		treatments I to IV had concentrations decreasing by a factor of 2 from treatment V until 0.125 mg Triclosan/kg. Additionally, samples were treated with the reference item dinoseb acetate. After application, the control, test item and reference item treated samples were adjusted to 45% of the soil MWC (i.e. 15.75 g water per 100 g dry soil). The samples were then incubated in the dark at 20 ± 2°C. The incubation temperature was monitored continuously. The moisture content of the samples was monitored on a weekly basis and moisture loss was compensated by the addition of purified water. Thereafter, the soil samples were thoroughly mixed. After incubation intervals, samples of treated and control soils were mixed with glucose, and glucose-induced respiration rates were measured. The amount of glucose concentration needed for maximum respiration response was determined in a preliminary test using a series of concentration of glucose. See also table A7_5_1_1-2.
3.3.3	Application of TS	An initial stock solution (I) was prepared by dissolving 58.21 g of test item in 3100 µL acetone. An aliquot of 400 µL of this solution was diluted to a final volume of 4000 µL with acetone (stock solution II). This solution corresponded to application solution V. Application solutions I to IV were prepared as follows: application solution I was prepared by taking 100 µL of stock solution II and diluting to 1000 µL with acetone. For application solutions II, III and IV, aliquots of 200 µL, 400 µL and 800 µL, were taken, respectively, and diluted to the corresponding volumes of 1000 µL, 1000 µl and 1600 µl with acetone. Volumes of 1 or 1.6 mL of the corresponding application solutions were added homogeneously to 15.0 g quartz sand (Merck 7712) and the solvent evaporated in a stream of nitrogen. The soil samples (150 g dry weight) received 1.5 g of the respective prepared quartz sand. See also see table A7_5_1_1-3.
3.3.4	Test conditions	See table A7_5_1_1-4.
3.3.5	Test parameter	Respiration can be regarded as a measure of the general turn-over of organic matter in soil. Respiration levels were determined by monitoring glucose-induced evolution of CO_2 resulting from microbial activity during short-term experiments (for approximately 30 hours each). In the present study, the influence of Triclosan on glucose-induced soil respiration was monitored in short-term experiments over an incubation period of 28 days.
3.3.6	Analytical parameter	CO_2 measurement
3.3.7	Duration of the test	28 days
3.3.8	Sampling	Respiration was determined for all dose levels at intervals of 0 (less than 3 hours) and 28 days after treatment.
3.3.9	Monitoring of TS concentration	No
3.3.10	Controls	Untreated control and positive control

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (02)

Annex Point IIA7.4

3.3.11	Statistics	The mean of individual values of the respiration experiments at the end of their respective incubation period were statistically evaluated by Dunnett's t-test (two-tailed, 5%) to find significant differences between control and treated samples (Dunnett, C.W., 1955 and 1964).	x
		4 RESULTS	
4.1	**Range finding test**	not performed	
4.1.1	Concentration	n.a.	
4.1.2	Effect data	n.a.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg Triclosan per kg dry soil.	
4.2.2	Actual concentrations of test substance	No measurements were done. The DT_{50} of Triclosan was determined in another test (see Study Summary 7.2.1 of Doc III-A): according to this test the actual concentration is expected to decline throughout the study.	
4.2.3	Growth curves	-	
4.2.4	Cell concentration data	-	
4.2.5	Concentration/ response curve	They are available in the report on page 24 and 25	
4.2.6	Effect data	No adverse effects were observed. See table A7_5_1_1-5 for detailed results.	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See table A7_5_1_1-5: includes data for all controls applied	
4.4	**Test with reference substance**	Yes, with dinoseb acetate	
4.4.1	Concentrations	Fortified sand was prepared at an amount corresponding to 3.76 mg dinoseb acetate per 1.5 g sand. Each soil sample (150 g dry weight) received 1.5 g of the prepared quartz sand. The amount of sand represented 10 g per kg soil.	
4.4.2	Results	The reference substance showed a clear influence on the microflora, thereby showing the sensitivity of the test system. See results in table A7_5_1_1-5.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The influence of Triclosan on soil microorganisms was determined by measuring the microbial respiration in soil Speyer 2.3 according to the OECD guideline 217. In the study, one fresh agricultural sandy loam soil, was moistened to 45% of its maximum water-holding capacity and incubated in the dark	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (02)

Annex Point IIA7.4

		at 20 ± 2°C following treatment with the test item. Control soil was not treated with the test item, but was incubated in parallel under identical conditions as for the treated soil. A geometric series of five concentrations (I to V) of Triclosan was tested for 28 days. Soil samples were treated with the test item at rates corresponding to concentrations of 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg Triclosan per kg dry soil.	
5.2	**Results and discussion**	After 28 days of incubation, the rate of respiration was 6.77 mg CO_2/h for the control and for the treated samples 6.13 mg (treatment I), 6.00 mg (II), 5.96 mg (III), 5.85 mg (IV) and 5.87 mg CO_2/h (V). In comparison to the untreated control, increasing deviations in respiration of -9.4% (treatment I), -11.3% (II), -12.0% (III), -13.5% (IV) and -3.2% (V) were observed for the treated samples. Thus, the test item had no detrimental effect on soil microbial respiration after 28 days of incubation with Triclosan, up to a concentration of 2.0 mg/kg dry soil. The reference item dinoseb acetate had a significant influence on the respiration (i.e., inhibition of -70.2% at day 28), thereby confirming the sensitivity of the test system and validity of the experimental design.	
5.2.1	NOEC	> 2 mg/kg dw soil	
5.2.2	EC_{10}	Not determined	
5.2.3	EC_{50}	Not determined	
5.3	Conclusion	The test item Triclosan had no detrimental effect on soil microbial respiration after 28 days of incubation, up to a concentration of 2.0 mg/kg dry soil.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	-	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2011
Materials and Methods	3.3.11: *Addition*: The Dixon-test, as reported by Sachs (1984) or Dixon (1953) was used to eliminate outliers in the respiration experiments.
Results and discussion	Agree with applicant's version.
Conclusion	5.3.2: *Addition*: The CO_2 production rates were calculated as an average value from the rate determinations during the constant initial respiration period (lag phase), while the OECD test guideline 216 prescribes to measure the total quantities of carbon dioxide released or oxygen consumed during the 12 first consecutive after glucose supplement and determine the mean respiration rates. The depth of sampling and the moisture contents during the test are not reported. The test concentrations used did not cover the range needed to determine ECx values.
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	-

Table A7_5_1_1-1: Properties of the soil sample

Criteria	Details
Nature	One soil sample (sandy loam soil: Speyer 2.3)
Source	[redacted]
Geographical reference on the sampling site	The soil was freshly sampled on August 04, 2006 and sieved through a 2-mm sieve by [redacted]
Data on the history of the site	The soil had not been subjected to any pesticide or organic fertilizer treatment in the sampling year and four former years. In the year 2002 it was fallow soil, from 2003 to 2005 pumpkins were planted and in 2006 the soil was uncultivated.
Use pattern	See above
Depth of sampling [cm]	Not specified
Sand / Silt / Clay content [% dry weight]	Classification USDA < 0.002 (clay) 9.2 0.002 - 0.05 (silt) 29.8 > 0.05 (sand) 61.0 Classification DIN < 0.002 (clay) 8.9 0.002 - 0.063 (silt) 31.5 > 0.063 (sand) 57.4
pH	pH ($CaCl_2$) 6.2 ± 0.3 pH (water) 7.4 (mean; was determined within this study)
Organic carbon content [% dry weight]	1.02 ± 0.15
Nitrogen content [% dry weight]	0.06
Cation exchange capacity [mval/100 g dry wt soil]	9 ± 1
Initial microbial biomass [mg C/kg dry wt soil]	134.2 (1.3 % of organic content)
Reference of methods	OECD Guidelines 217 (2000)
Collection / storage of samples	The soil was freshly sampled on August 04, 2006 and sieved through a 2-mm sieve
Preparation of inoculum for exposure	The soil was filled into incubation flasks and equilibrated at 20 ± 2°C in the dark. The soil moisture content was adjusted to just below 45% of the maximum water holding capacity (MWC) prior to application.
Pretreatment	n.a.

Table A7_5_1_1-2: Test system

Criteria	Details
Culturing apparatus	After mixing, soil was incubated in 1-litre incubation flasks closed with cotton wool plugs.
Number of vessels / concentration	3
Aeration device	Not specified
Measuring equipment	IR gas analyser. The concentration of CO_2/L air was used to calculate the volume of CO_2 evolved/h/kg dry soil.
Test performed in closed vessels	Yes

Table A7_5_1_1-3: Application of test substance and sampling

Criteria	Details
Application procedure	Addition of premixtures in a carrier and mixing the carrier with native soil.
Carrier	Triclosan was dissolved in acetone and the Triclosan acetone solution was added to quartz sand and the acetone was then evaporated under a stream of nitrogen.
Concentration of liquid carrier [% v/v]	See section 3.3.3
Liquid carrier control	no
Sampling procedure	Respiration was determined in the beginning and 28 days after treatment.

Table A7_5_1_1-4: Test conditions

Criteria	Details
Organic (inorganic) substrate	Addition of glucose
Incubation temperature	20 ± 2 °C
Soil moisture	Adjusted to 45% of the maximum soil water holding capacity (MWC of soil = 34.90 ± 3.0%).
Method of soil incubation	Bulk
Aeration	-

Table A7_5_1_1-5: Influence of the test item and dinoseb acetate on glucose-induced short-term respiration of soil Speyer 2.3.

	Incubation time [days]	Respiration rates [mg CO_2/h per kg dry soil]						
		Replicates						
		a	b	c	Mean	[SD]	%SD	% Deviation from control
Control	0	7.01	6.98	6.87	6.95	0.07	1.0	-
	28	7.08	6.67	6.56	6.77	0.27	4.0	-
Treatment I	0	6.66	6.65	6.53	6.61*	0.07	1.1	-4.9
	28	6.35	6.09	5.96	6.13*	0.20	3.3	-9.4
Treatment II	0	6.46	6.54	6.45	6.48*	0.05	0.8	-6.8
	28	5.98	6.08	5.94	6.00*	0.08	1.3	-11.3
Treatment III	0	6.46	6.42	6.52	6.47*	0.05	0.8	-7.0
	28	5.96	5.92	5.99	5.96*	0.03	0.6	-12.0
Treatment IV	0	6.15	6.28	6.29	6.24*	0.08	1.2	-10.3
	28	5.90	5.84	5.81	5.85*	0.04	0.7	-13.5
Treatment V	0	6.10	6.15	6.10	6.12*	0.03	0.5	-12.0
	28	5.79	5.95	5.87	5.87*	0.08	1.3	-13.2
Dinoseb acetate	0	4.04	3.75	3.85	3.88*	0.15	3.8	-44.3
	28	2.00	1.95	2.10	2.02*	0.08	3.8	-70.2

*: Value is significantly different from the control (Dunnett-test, two-sided, $\alpha = 0.05$)

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (03)

Annex Point IIA7.4

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2007b): The Effects of Triclosan on Soil Nitrification. [redacted] Report No. [redacted] date: 2007-02-21 (unpublished).
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	-
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD Guideline 216, 2000; Under consideration of: EPPO, Chapter 7, Soil Microflora, Volume 24, No. 1, 1994
2.2	**GLP**	Yes
2.3	**Deviations**	No

3 MATERIALS AND METHODS

3.1	**Test material**	**Triclosan**
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	-
3.1.3	Purity	99.3 %
3.1.4	Composition of Product	-
3.1.5	Further relevant properties	-
3.1.6	Method of analysis	**Microbial Biomass determination**: Bulk samples of untreated soil were prepared for microbial biomass analysis by sieving and adjusting the moisture content to 45% of its MWC. Seven subsamples of 40 g wet soil each (corresponding to 34.6 g dry soil) were mixed with different concentrations of glucose and talc. The talc served as an inert ingredient to improve the homogeneous distribution of the glucose in the soil. For the biomass determination, only those glucose concentrations providing the three highest initial and constant CO_2 production rates (substrate saturation conditions) were considered (mixtures 3-5). The CO_2 production rates were calculated as an average value (V_{CO2}) from the rate determinations during the constant initial respiration period (lag phase). The CO_2 evolved was measured with an infrared (IR) gas analyser. The CO_2 evolved from each sample was calculated based on the accumulation time, the flow rate, the measurement period, and the concentration of carbon dioxide in the outlet air stream.

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (03)

Annex Point IIA7.4

		Nitrite (NO_2^-) Analysis: The determination of nitrite is based on a colorimetric method. In this method, the nitrite ion reacts with sulfanilamides under acidic conditions to yield a diazo compound which is coupled with N-1-naphthylethylene-diamine dihydrochloride to form a soluble dye. The concentration is measured colorimetrically at a wavelength of 550 nm. The limit of quantitation (LOQ) is 0.02 mg nitrogen-nitrite/L extract, corresponding to 0.09 mg nitrite/kg dry soil. **Nitrate (NO_3^-) Analysis:** Nitrate is reduced to nitrite in a cadmium reductor. The nitrite is then determined as described above. The method determines the sum of free nitrite and nitrate simultaneously [(NO_2^-) + (NO_3^-)]. Hence, the NO_3^- concentrations are calculated by subtracting NO_2^- as measured above from the [(NO_2^-) + (NO_3^-)] concentration. The limit of quantitation (LOQ) is 0.1 mg nitrogen-nitrate/L extract, corresponding to 0.55 mg nitrate/kg dry soil.
3.2	**Reference substance**	Yes, 2-Chloro-6-trichloromethylpyridine (common name: Nitrapyrin PESTANAL®).
3.2.1	Method of analysis for reference substance	Not analysed, only nitrite and nitrate
3.3	**Testing procedure**	
3.3.1	Soil sample / inoculum / test organism	see table A7_5_1_1-1
3.3.2	Test system	Soil samples (amended with lucerne meal) of approximately 150 g dry weight were set-up in 1-litre incubation flasks stoppered with cotton wool plugs. Control samples were not treated with the test item. Treatment V samples had a concentration of 2.0 mg a.i./kg dry soil. Samples of treatments I to IV had concentrations decreasing by a factor of 2 from treatment V until 0.125 mg a.i./kg. Additionally, samples were treated with the reference item nitrapyrin. After application, the control, test item and nitrapyrin treated samples were adjusted to 45% of the soil MWC (i.e. 15.57 g water per 100 g dry soil). The samples were then incubated in the dark at 20 ± 2°C. The incubation temperature was monitored continuously. The moisture content of the samples was monitored on a weekly basis and moisture loss was compensated by the addition of purified water. After addition of water, the soil samples were thoroughly mixed. See table A7_5_1_1-2.
3.3.3	Application of TS	An initial stock solution (I) was prepared by dissolving 58.21 g of test item in 3100 µL acetone. An aliquot of 400 µL of this solution was diluted to a final volume of 4000 µL with acetone (stock solution II). This solution corresponded to application solution V. Application solutions I to IV were prepared as follows: application solution I was prepared by taking 100 µL of stock solution II and diluting to 1000 µL with acetone. For application solutions II, III and IV, aliquots of 200 µL, 400 µL and 800 µL, were taken, respectively, and diluted to the corresponding volumes of 1000 µL, 1000 µl and 1600

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (03)

Annex Point IIA7.4

		µl with acetone.	x
		Volumes of 1 or 1.6 mL (see table below for details) of the corresponding application solutions were added homogeneously to 15.0 g quartz sand (Merck 7712) and the solvent evaporated in a stream of nitrogen. The soil samples (150 g dry weight) received 1.5 g of the respective prepared quartz sand.	
		See also table A7_5_1_1-3.	
3.3.4	Test conditions	see table A7_5_1_1-4	
3.3.5	Test parameter	The microbial conversion of organic nitrogen to nitrate is a multi-step process. In a primary reaction, soil organic matter is mineralised to ammonia (ammonification, mineralisation). A wide range of possible sinks of ammonia are reported (Paul & Clark, 1988), e.g. immobilisation by soil microorganisms by incorporation and formation of amino acids, and ion exchange or adsorption with soil constituents. Furthermore, ammonia is converted via nitrite to nitrate, a process which is designated as nitrification. This step represents the second step in organic matter conversion. The nitrification processes are considered to be important for soil fertility.	
3.3.6	Analytical parameter	Nitrate and nitrite measurement	
3.3.7	Duration of the test	28 days	
3.3.8	Sampling	The level of nitrification was determined for all dose levels at intervals of 0 (less than 3 hours) and 28 days after treatment.	
		The concentration of nitrite and nitrate was determined for each sampling interval in a 2 M KCl extract of the soil sample.	
3.3.9	Monitoring of TS concentration	No	
3.3.10	Controls	Untreated control and positive control	
3.3.11	Statistics	The mean of individual values of the nitrification experiments at the end of their respective incubation period were statistically evaluated by Dunnett's t-test (two-tailed, 5%) to find significant differences between control and treated samples (Dunnett, C.W., 1955 and 1964).	x
		4 RESULTS	
4.1	**Range finding test**	not performed	
4.1.1	Concentration	n.a.	
4.1.2	Effect data	n.a.	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg Triclosan per kg dry soil	
4.2.2	Actual concentrations of test substance	No measurements were done. The DT_{50} of Triclosan was determined in another test (see Study Summary 7.2.1 of Doc III-A): according to this test the actual concentration is expected to decline throughout the study.	

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (03)

Annex Point IIA7.4

4.2.3	Growth curves	-	
4.2.4	Cell concentration data	-	
4.2.5	Concentration/ response curve	A graphical presentation of NO_3^- values during the test period for each test concentration is shown in Figure 3 of the report.	
4.2.6	Effect data	No adverse effects were observed. See table A7_5_1_1-5 and A7_5_1_1-6 for detailed results.	
4.2.7	Other observed effects	-	
4.3	**Results of controls**	See tables A7_5_1_1-5 and A7_5_1_1-6: include data for all controls applied.	
4.4	**Test with reference substance**	Yes, with 2-Chloro-6-trichloromethylpyridine (common name: Nitrapyrin PESTANAL®).	
4.4.1	Concentrations	Soil samples were treated with 1.50 mg nitrapyrin/150 g dry soil corresponding to 5 mg nitrapyrin/kg dry soil.	x
4.4.2	Results	The reference substance showed a clear influence on the microflora, thereby showing the sensitivity of the test system. See results in tables A7_5_1_1-5 and A7_5_1_1-6.	

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The influence of the test item Triclosan on the nitrification of lucerne meal in soil was investigated in soil Speyer 2.3 according to OECD Guideline 216 and EPPO, Chapter 7, Soil Microflora, Volume 24, No. 1, 1994. In the study, one fresh agricultural sandy loam soil, was moistened to 45% of its maximum water-holding capacity and incubated in the dark at 20 ± 2°C following treatment with the test item. Control soil was not treated with the test item, but was incubated in parallel under identical conditions as for the treated soil. A geometric series of five concentrations (I to V) of Triclosan was tested. Soil samples were treated with the test item at rates corresponding to concentrations of 0.125 mg, 0.25 mg, 0.5 mg, 1.0 mg and 2.0 mg Triclosan per kg dry soil.
5.2	**Results and discussion**	For the control, the mean initial concentration of nitrite was 0.645 mg NO_2^-/kg dry soil and for treatments I to V it was 0.606 mg/kg (treatment I), 0.637 mg/kg (II), 0.617 mg/kg (III), 0.620 mg/kg (IV), and 0.661 mg/kg (V), respectively. On day 28, no nitrite was detected for all treatments. The nitrate concentration on Day 28 was 116.3 mg NO_3-/kg dry soil for the control and 105.4 mg/kg, 94.6mg/kg, 105.0 mg/kg, 110.1 mg/kg and 107.4 mg/kg mg NO_3-/kg dry soil for the five treatment rates I to V, respectively. The calculated deviations to control were -9.3%, -18.6%, -9.7%, -5.4% and -7.6%, respectively. A deviation of -7.6% from the control (nitrate) was obtained for the highest concentration tested, i.e. 2.0 mg a.i./kg dry soil after 28 days of

Section A7.5.1.1 Inhibition to microbial activity (terrestrial) (03)

Annex Point IIA7.4

		incubation indicating that Triclosan had no detrimental effect on the nitrification up to and including this concentration. Furthermore, additional samples were treated with a reference compound, nitrapyrin (at a rate of 5 mg/kg soil), to show the sensitivity of the test system and method used. This concentration corresponds to a field rate of 3.75 kg nitrapyrin/ha. After 28 days of incubation, the calculated deviation to control was -82.4% for nitrate formation.	
5.2.1	NOEC	> 2 mg/kg dw soil	
5.2.2	EC_{10}	Not determined	
5.2.3	EC_{50}	Not determined	
5.3	Conclusion	A deviation of -7.6% from the control (nitrate) was obtained for the highest concentration tested, i.e. 2.0 mg a.i./kg dry soil after 28 days of incubation indicating that Triclosan had no detrimental effect on the nitrification up to and including this concentration.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	-	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	June 2011
Materials and Methods	3.3.3: *Deletion*: (see table below for detail) 3.3.11: *Addition*: The Dixon-test, as reported by Sachs (1984) or Dixon (1953) was used to eliminate outliers in the nitrification experiments.
Results and discussion	4.4.1: Replace “Soil samples were treated with 1.50 mg nitrapyrin/150 g dry soil corresponding to 5 mg nitrapyrin/kg dry soil.” by “Soil samples (150 g dry weight) received 1.5 g of the prepared quartz sand containing 3.76 mg nitrapyrin, corresponding to 25 mg nitrapyrin/kg dry soil.”
Conclusion	5.3.2: *Addition*: The depth of sampling, the carbon content and the used sieve size for the substrate luzerne meal, and the moisture contents during the test are not reported. The test concentrations used did not cover the range needed to determine ECx values.
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Also microbial biomass determination is described in the test report.

Table A7_5_1_1-1: Properties of the soil sample

Criteria	Details
Nature	One soil sample (sandy loam soil: Speyer 2.3)
Source	[redacted]
Geographical reference on the sampling site	The soil was freshly sampled on August 04, 2006 and sieved through a 2-mm sieve by [redacted]
Data on the history of the site	The soil had not been subjected to any pesticide or organic fertilizer treatment in the sampling year and four former years. In the year 2002 it was fallow soil, from 2003 to 2005 pumpkins were planted and in 2006 the soil was uncultivated.
Use pattern	See above
Depth of sampling [cm]	Not specified
Sand / Silt / Clay content [% dry weight]	Classification USDA < 0.002 (clay) 9.2 0.002 - 0.05 (silt) 29.8 > 0.05 (sand) 61.0 Classification DIN < 0.002 (clay) 8.9 0.002 - 0.063 (silt) 31.5 > 0.063 (sand) 57.4
pH	pH ($CaCl_2$) 6.2 ± 0.3 pH (water) 7.4 (mean; was determined within this study)
Organic carbon content [% dry weight]	1.02 ± 0.15
Nitrogen content [% dry weight]	0.06
Cation exchange capacity [mval/100 g dry wt soil]	9 ± 1
Initial microbial biomass [mg C/kg dry wt soil]	134.2 (1.3 % of organic content)
Reference of methods	OECD Guidelines 216 (2000)
Collection / storage of samples	The soil was freshly sampled on August 04, 2006 and sieved through a 2-mm sieve
Preparation of inoculum for exposure	The soil was filled into incubation flasks and equilibrated at 20 ± 2°C in the dark. The soil moisture content was adjusted to just below 45% of the maximum water holding capacity (MWC) prior to application. Furthermore, all samples were amended with lucerne meal as a source of organic nitrogen. The lucerne meal contained approximately 3% nitrogen.
Pretreatment	n.a.

Table A7_5_1_1-2: Test system

Criteria	Details
Culturing apparatus	After mixing, soil was incubated in 1-litre incubation flasks closed with cotton wool plugs.
Number of vessels / concentration	3
Aeration device	Not specified
Measuring equipment	The concentrations of nitrite and nitrate were measured colorimetrically using a Flow Injection Analyser (AutoAnalyzer 3, Bran+Luebbe).
Test performed in closed vessels	Yes

Table A7_5_1_1-3: Application of test substance and sampling

Criteria	Details
Application procedure	Addition of premixtures in a carrier and mixing the carrier with native soil.
Carrier	Triclosan was dissolved in acetone and the Triclosan acetone solution was added to quartz sand and the acetone was then evaporated under a stream of nitrogen.
Concentration of liquid carrier [% v/v]	See 3.3.3
Liquid carrier control	no
sampling procedure	Nitrification was determined in the beginning and 28 days after treatment.

Table A7_5_1_1-4: Test conditions

Criteria	Details
Organic (inorganic) substrate	The soil samples were amended with 0.8 g lucerne meal (nitrogen content approximately 3%) after application.
Incubation temperature	20 ± 2 °C
Soil moisture	Adjusted to 45% of the maximum soil water holding capacity by adding purified water (MWC = 34.90 ± 3.0 %).
Method of soil incubation	Bulk
Aeration	-

Table A7_5_1_1-5: Influence of the test item and nitrapyrin on nitrite formation after amendment of lucerne meal.

	Incubation time [days]	Nitrite [mg NO_2^-/ kg dry soil]						
		Replicates a	b	c	Mean	SD	%SD	% Deviation from control
Control	0	0.637	0.649	0.649	0.645	0.007	1.1	-
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	-
TI	0	0.600	0.612	0.587	0.606*	0.012	2.0	-4.7
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.
TII	0	0.641	0.633	0.637	0.637	0.004	0.6	<0.1
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.
TIII	0	0.620	0.616	0.616	0.617*	0.002	0.4	-3.1
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.
TIV	0	0.616	0.624	0.620	0.620*	0.004	0.7	-3.1
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.
TV	0	0.665	0.669	0.649	0.661*	0.011	1.6	3.1
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.
Nitrapyrin	0	0.452	0.448	0.452	0.450*	0.002	0.5	-29.7
	28	<0.086	<0.086	<0.086	<0.086	<0.001	n.a.	n.a.

Table A7_5_1_1-6: Influence of the test item and nitrapyrin on nitrate formation after amendment of lucerne meal.

	Incubation time [days]	Nitrate [mg NO_3^-/ kg dry soil]						
		Replicates a	b	c	Mean	SD	%SD	% Deviation from control
Control	0	99.4	99.8	100.1	99.8	0.3	0.3	-
	28	115.2	118.4	115.3	116.3	1.8	1.6	-
TI	0	99.2	99.5	96.6	98.5	1.5	1.5	-1.3
	28	106.6	104.3	105.4	105.4*	1.1	1.1	-9.3
TII	0	103.3	102.6	101.1	102.4*	1.1	1.1	2.6
	28	95.1	94.0	94.9	94.6*	0.6	0.6	-18.6
TIII	0	94.0	94.8	95.2	94.7*	0.6	0.7	-5.1
	28	101.1	102.2	111.6	105.0*	5.8	5.5	-9.7
TIV	0	94.2	96.2	95.9	95.4*	1.1	1.1	-4.4
	28	112.6	108.6	108.9	110.1*	2.2	2.0	-5.4
TV	0	95.6	97.8	96.3	96.6*	1.1	1.1	-3.2
	28	108.6	106.6	107.1	107.4*	1.0	0.9	-7.6
Nitrapyrin	0	94.8	95.3	97.3	95.8*	1.3	1.4	-4.0
	28	20.7	20.0	20.5	20.4*	0.4	1.8	-82.4

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII.3.2

Eisenia fetida

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](2001): Acute Toxicity of [redacted] to the Earthworm, *Eisenia fetida*. [redacted] Report No [redacted] date: 2001-07-03 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	OECD Guidelines for Testing of Chemicals, Section 2, No. 207 (1984); Commission Directive 87/302/EEC, L 133 Part C, pp.95-98, (Edition 1988)	
2.2	**GLP**	Yes	
2.3	**Deviations**	None	
		3 METHOD	
3.1	**Test material**	[redacted](DCPP)	
3.1.1	Lot/Batch number	Batch no. [redacted]	
3.1.2	Specification	As given in section 2 of the dossier	
3.1.3	Purity	Not given	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	-	
3.2	**Reference substance**	Yes; 2-chloracetamide	
3.2.1	Method of analysis for reference substance	No chemical analysis of the test substance in the test substrate was performed.	
3.3	**Testing procedure**		
3.3.1	Preparation of the test substance	No solvent was used. 40 g of a stock mixture of test item and fine quartz sand was made up for each test concentration.	
3.3.2	Application of the test substance	Since the test item was not soluble at the desired concentrations in deionised water, an aliquot of the stock mixture was mixed thoroughly into fine quartz sand and then added to the dry blended constituents of the formulated soil.	x

Section A7.5.1.2 Earthworm, acute toxicity test
Annex Point IIIA XIII.3.2

Eisenia fetida

3.3.3	Test organisms	*Eisenia fetida*, see Table A7_5_1_2-2
3.3.4	Test system	See table A7_5_1_2-3
3.3.5	Test conditions	See table A7_5_1_2-4
3.3.6	Test duration	14 days
3.3.7	Test parameter	Mortality, weight alteration of the survivors and sublethal effects. For soil quality criteria, temperature, soil moisture, light intensity and pH were determined.
3.3.8	Examination	**Soil quality:** Soil water content of each test concentration and the control was measured at the start and the end of the test. An approximate 10 g sample of wet soil was taken and the moisture content was determined by weighing a defined sub-sample before and after. At test termination, a sub-sample of soil (approx. 3 g) from each of the four replicates was taken and pooled for the determination of moisture content of the soil. At test initiation, the pH of each batch of soil was measured. Temperature in the environmental chamber was recorded continuously. Light intensity was measured at the beginning of the test. **Biological observations:** At test initiation, the weight of each earthworm was determined individually and the weight of ten earthworms for each replicate was recorded. At test initiation and after 7 days of exposure, the time taken by the earthworms to burrow into the soil after initial placing on the soil surface was recorded. Observations of burrowing time were made up to two hours following the positioning of the test organisms on the soil surface. After 7 and 14 days of exposure, observations on mortality and perceivable sublethal effects, such as abnormal behaviour or flaccidity were performed. Dead organisms, if any, were removed from the test soil. At test termination, the weight of the surviving earthworms for each replicate of each test concentration was recorded.
3.3.9	Monitoring of test substance concentration	Not performed
3.3.10	Statistics	The statistical analysis of the data was performed with a statistical program (Ratte H T, 1995a). The LC_{50} value at 14 days and the confidence limits were determined by binomial distribution. For the determination of the NOEC in regard to average live weight of test organisms, Dunnett multiple sequential test procedure was performed (Ratte H T, 1995b).

4 RESULTS

4.1	**Filter paper test**	Not performed
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII.3.2

Eisenia fetida

4.2	**Soil test**	
4.2.1	Initial concentrations of test substance	No range finding test was carried out. Nominal concentrations: 95, 171, 309, 556 and 1000 mg test item / kg dry weight of soil.
4.2.2	Effect data (Mortality)	See table A7_5_1_2-5 and A7_5_1_2-9
4.2.3	Concentration / effect curve	No concentration / effect curve is given in the report
4.2.4	Other effects	See table A7_5_1_2-6, A7_5_1_2-7 and A7_5_1_2-8
4.3	**Results of controls**	
4.3.1	Mortality	No mortality of earthworms was observed at day 7 and day 14 of the test in the control
4.3.2	Number/ percentage of earthworms showing adverse effects	No increased burrowing time or sublethal effects, such as abnormal behaviour or flaccidity or other symptoms were observed in the control.
4.3.3	Nature of adverse effects	-
4.4	**Test with reference substance**	2-chloroacetamide. The test was carried out in April 2001.
4.4.1	Concentrations	Not reported
4.4.2	Results	The LC_{50} was 34 mg/kg (30 - 38 mg/kg 95% confidence limits).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The objective of this study was the estimation of the acute toxicity of DCPP to the earthworm, *Eisenia fetida*. Earthworms were exposed to a range of concentrations (95, 171, 309, 556 and 1000 mg test item / kg dry weight of soil) of the test item in formulated soil over a 14 day period according to OECD guideline 207. Mortality, weight alteration of surviving animals, and sublethal effects were assessed after 7 and 14 days and the LC_{50} (median lethal concentration) estimated. Burrowing time after 7 days. Chloracetamide is the recommended reference item for acute earthworm toxicity tests. .
5.2	**Results and discussion**	No **mortality** of earthworms was observed at day 7 and day 14 of the test, in the control and at test concentrations of 95, 171 and 309 mg of DCPP/kg dry weight of soil. At a test concentration of 556 mg test item/kg dry weight of soil, 2.5% mortality of earthworms had occurred after an exposure period of 7 days, increasing to 20% mortality after 14 days of the test. 33% mortality of earthworms occurred at the highest test concentration of 1000 mg DCPP/kg dry weight of soil after 7 days of exposure, increasing to 100% mortality at the end of the 14 day test period. No **sublethal effects**, such as abnormal behaviour or flaccidity or other

Section A7.5.1.2 Earthworm, acute toxicity test

Annex Point IIIA XIII.3.2

Eisenia fetida

		symptoms were observed in the control and treatment concentrations of 95 and 171 mg of DCPP/kg dry weight of soil at day 7 and 14 of the test. At a test concentration of 309 mg/kg dry weight of soil, no sublethal effects were observed at 7 days of the test, but light sublethal symptoms were recorded after 14 days of exposure, the earthworms showed abnormal contractions of their bodies and open wounds in one of the replicates. At a test concentration of 556 mg test item/kg dry weight of soil, light sublethal effects were observed on day 7 of the test which increased to severe symptoms in the surviving earthworms after 14 days. At this test concentration the symptoms observed were open wounds, abnormal contractions of the earthworms‘ bodies as well as abnormal behaviour, the earthworms were climbing the walls of the test vessel after placing them on the soil surface after 7 days of the test instead of returning to the soil. On day 14 of the test, it was also recorded that worms were very thin and were found on the soil surface. At a test concentration of 1000 mg test item/kg dry weight of soil, severe sublethal effects (symptoms as described above at 556 mg/kg) were recorded in the surviving earthworms. The **average live weight** of the earthworms in the control and test concentrations of 95, 171, 309 and 556 mg DCPP/kg dry weight of soil, changed to a weight of 99, 95, 98, 86 and 76% of the initial weight during the 14 days of exposure, respectively. The average weight change (in %) in comparison to the controls was significantly different (Dunnett-test; α. = 0.05, one-sided, smaller) in test concentrations of 309 and 556 mg/kg dry weight of soil. Hence the NOEC (14d) regarding earthworm weight was determined to be 171 mg DCPP/kg dry weight of soil. No increased **burrowing time** was observed in the control and any of the test concentrations at the start of the test. After 7 days of exposure, no increased burrowing time was observed in the control and at test concentrations of 95, 171 and 309 mg DCPP /kg dry weight of soil. At a test concentration of 556 mg/kg, increased burrowing time was recorded at 7 days of the test, earthworms had only entered the soil within 45 minutes of initially placing them on the soil surface. At the highest test concentration of 1000 mg/kg, increased burrowing time was recorded at 7 days of the test, with the surviving earthworms still remaining outside the soil after 2 hours of initially placing them on the soil surface.	
5.2.1	LC_0	309 mg a.s./kg dry weight soil	
5.2.2	LC_{50}	693 mg a.s./ kg dry weight soil (95% C.I.: 556 – 1000 mg a.s./ kg)	
5.3	**Conclusion**	Validity criteria according to the OECD guideline 207 are fulfilled, test results can be considered reliable (see table A7_5_1_2-10).	
5.3.1	Other Conclusions	-	
5.3.2	Reliability	1	
5.3.3	Deficiencies	Purity not given in the report	x

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	April 2011
Materials and Methods	*Deletion* in Table A7_5_1_2-1: Delete term “yes” in box “Dispersion”.
Results and discussion	Agree with applicant's version.
Conclusion	5.3.3: *Addition* after term “report”: “but meanwhile company confirmed a purity of 99.5% for the used batch. No plotting of the concentration/response curve is provided. At 309 mg/kg light symptoms are described in the text, but not mentioned in table A7_5_1_2-6. The water content is slightly above 42%: At the start of the test, the moisture content of the formulated soil ranged from 43.9 to 45.3% of the dry weight of soil and from 42.3 to 42.9 at the end of the test. No information regarding water solubility or vapour pressure is provided in the report.”
Reliability	1, as deficiencies are of minor nature and a purity of 99.5% is meanwhile confirmed.
Acceptability	Acceptable for environmental risk assessment with amendments given above
Remarks	In those details where the test method according to Directive 87/302/EEC differs from OECD No. 207 (1984) the Directive 87/302/EEC was followed.

Table A7_5_1_2-1: Preparation of TS solution

Criteria	Details
Type and source of dilution water	No water used, quartz sand was used instead
Alkalinity / Salinity	-
Hardness	-
pH	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	-
In case of the use of an organic solvent	
Dispersion	Yes
Vehicle	-
Concentration of vehicle	-
Vehicle control performed	-
Other procedures	-

Table A7_5_1_2-2: Test organisms

Criteria	Details
Species/strain	*Eisenia fetida*
Source of the initial stock	Holding and source of the earthworms were obtained from in-house cultures at the organisms Ecotox Center, [redacted] they were bred. Originally earthworms were supplied by SARL [redacted]
Culturing techniques	They were bred under standardised conditions in a mixture of peat and cattle manure. Originally earthworms were supplied by SARL Moulin, F-60510 Litz. Earthworms were not treated against any disease during holding.
Age/weight	Adult earthworms of an approximate age of 3 months and a wet mass between 314 and 378 mg were used in the test.
Pre-treatment	Procedure prior to test before exposure earthworms were conditioned in the same formulated soil substrate as used in the test for 24 hours. Just prior to test initiation earthworms were quickly washed, weighed individually and the weight of ten animals recorded. Exposure started when test organisms were placed on the soil surface and allowed to enter the soil.

Table A7_5_1_2-3: Test system

Criteria	Details
Artificial soil test substrate	Formulated Soil (components based on % dry weight) - 10% Sphagnum peat - 20% Kaolin clay (kaolinite content >30%) - 69% Industrial quartz sand (>50% of particles are 50 - 200 µm) - approximately 1% calcium carbonate to reach a pH of 6.0 ± 0.5 (in this test 1.2 - 1.4% of $CaCO_3$ were added to the formulated soil substrate to reach the desired pH) The water content was adjusted to approximately 40% of the dry weight of the soil by the addition of deionised water.
Test mixture	The total amount of soil needed for the 4 replicates per treatment level was prepared in one batch, hence 2100 g of dry soil corresponding to 2940 g of wet soil was made up. Mixing of the soil took place in a BEAR Varimixer RN 20. To reach a test concentration of 95 mg/kg of dry weight of soil, 199.5 mg of DCPP was mixed thoroughly into the sand and then added to the dry blended constituents of the formulated soil. For test concentrations of 171, 309, 556 and 1000 mg/kg dry weight of soil, 359.3, 649.1, 1167.9 and 2099.6 mg of test item, respectively, were pre-mixed with sand and then added to 2060 g (dry weight) of formulated soil.
Size, volume and material of test container	1.5 L glass bottling jars filled with 700 g of moist soil (500 g dry weight) which were covered by glass lids allowing for gas exchange and minimising evaporation.
Amount of artificial soil (kg)/ container	Per test beaker, 700 g wet artificial soil corresponding to 500 g dry soil was added.
Nominal levels of test concentrations	95, 171, 309, 556 and 1000 mg test item / kg dry weight of soil
Number of replicates/concentration	4
Number of earthworms/test concentration	40
Number of earthworms/container	10
Light source	Continuous light (455 Lux: mean of four measurements: 400, 520, 500, 400 Lux)
Test performed in closed vessels due to significant volatility of test substrate	Yes

Table A7_5_1_2-4: Test conditions

Criteria	Details
Test temperature	19.0 - 19.8°C
Moisture content	Approximately 40% of the dry weight of the soil (at the start of the test, the moisture content of the formulated soil ranged from 43.9 to 45.3% of the dry weight of soil and from 42.3 to 42.9% at the end of the test)
pH	At test initiation: 5.9 to 6.5
Adjustment of pH	Soil contained 1% calcium carbonate to reach a pH of 6.0 ± 0.5
Light intensity / photoperiod	Continuous light (455 Lux)
Relevant degradation products	Degradation products were not investigated in this study

Table A7_5_1_2-5: Mortality of earthworms after 7 and 14 days

Nominal concentration	Cumulative mortality			
	Day 7		**Day l4**	
(mg/kg of dry weight of soil)	Number of dead animals	%	Number of dead animals	%
Control	0	0	0	0
95	0	0	0	0
171	0	0	0	0
309	0	0	0	0
556	1	2.5	8	20
1000	13	33	40	100

Table A7_5_1_2-6: Sublethal effects observed after 7 and 14 days

Nominal conc. (mg/kg of dry weight of soil)	Sublethal effects					
	Day 7			Day 14		
	Abnormal behaviour	Flaccidity	Others (open wounds)	Abnormal behaviour	Flaccidity	Others (open wounds)
Control	0	0	0	0	0	0
95	0	0	0	0	0	0
171	0	0	0	0	0	0
309	0	0	0	0	0	1
556	1*	1*	1*	3*	3*	3*
1000	3*	3*	3*	**	**	**

0 no symptoms 1 light symptoms 2 moderate symptoms 3 severe symptoms
* some worms dead ** all worms dead

Table A7_5_1_2-7: Weight of test organisms at the start and end of the test

Nominal conc. (mg/kg of dry weight of soil)	No. of test vessel	Average live weight of test organisms (mg)		Weight difference compared to start of test		
		Start of test	End of test	mg/worm	%	Average (%)
Control	1	322	306	-16	95	99
	2	360	332	-28	92	
	3	341	338	-3	99	
	4	327	356	29	109	
95	5	314	312	-2	99	95
	6	342	333	-9	97	
	7	349	317	-32	91	
	8	345	324	-21	94	
171	9	338	333	-5	99	98
	10	355	339	-16	95	
	11	326	324	-2	99	
	12	329	318	-11	97	
309	13	364	301	-63	83	86*
	14	339	311	-28	92	
	15	345	287	-58	83	
	16	332	289	-43	87	
556	17	329	265	-64	81	76*
	18	351	260	-91	74	
_____	19	334	256	-78	77	
	20	322	234	-88	73	
1000	21	333	**	-	-	-
	22	378	**	-	-	
	23	336	**	-	-	
	24	378	**	-	-	

* Significant (Dunnett-test; o = 0.05, one-sided, smaller) weight change (in %) in comparison to the control

Table A7_5_1_2-8: Burrowing time observed at test start and day 7 of the test

Nominal conc. (mg/kg of dry weight of soil)	No. of test vessel	Burrowing time (time taken by earthworms to burrow into soil after initial placing on the soil surface; observations were made up to 2 hours)	
		Start of test	Day 7
Control	1	within 15 min	within 15 min
	2	within 15 min	within 15 min
	3	within 15 min	within 15 min
	4	within 15 min	within 15 min
95	5	within 15 min	within 15 min
	6	within 15 min	within 15 min
	7	within 15 min	within 15 min
	8	within 15 min	within 15 min
171	9	within 15 min	within 15 min
	10	within 15 min	within 15 min
	11	within 15 min	within 15 min
	12	within 15 min	within 15 min
309	13	within 15 min	within 15 min
	14	within 15 min	within 15 min
	15	within 15 min	within 15 min
	16	within 15 min	within 15 min
556	17	within 15 min	within 45 min*
	18	within 15 min	within 45 min
	19	within 15 min	within 45 min
	20	within 15 min	within 45 min
1000	21	within 15 min	after 2 hours earthworms still remain an soil surface *
	22	within 15 min	after 2 hours earthworms still remain an sail surface *
	23	within 15 min	after 2 hours earthworms still remain an soil surface
	24	within 15 min	after 2 hours earthworms still remain an soil surface*

*some worms dead

Table A7_5_1_2-9: Effect data based on nominal concentrations

	7 d [mg/kg dw soil]	14 d [mg/kg dw soil]
LC_0	309	309
LC_{50}	>1000	693 (95 % c.l. = 556-1000)

Table A7_5_1_2-10: Validity criteria for acute earthworm test according to OECD guideline 207

	Fulfilled	Not fulfilled
Mortality of control animals < 10%	**X**	

Section A7.5.1.3 **Annex Point IIIA XIII.3.4**	**Terrestrial plant toxicity**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	Since DCPP containing biocidal products are used indoor, only sewage treatment plants will be the receiving compartment for DCPP residues. However, sewage sludge might be applied to soil as fertilizer. Therefore, soil can be contaminated indirectly. Since the compound is inherent biodegraded in the sewage treatment plant, residues of DCPP in the sewage sludge are low and thus, only low amounts of the active substance might reach the soil compartment. A study with terrestrial plants exposed to the structurally similar active substance triclosan (CAS-No. 3380-34-5) is available. The phenol ring of triclosan and DCPP is identical. Furthermore, since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that triclosan has a higher toxicity to organisms than DCPP. Thus, it is considered justified to bridge the results of the study performed with triclosan to DCPP.	x x x
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	June 2011	
Evaluation of applicant's justification	Exchange "Since DCPP containing biocidal products are used indoor, only sewage treatment plants will be the receiving compartment for DCPP residues." by "According to the Intended Use (Doc. II-B) no direct exposure to surface water, only indirect exposure via STP is possible." Exchange "inherent biodegraded" by "eliminated to a high degree". Exchange "A study" by "Studies" and "is" by "are".	
Conclusion	Agree with applicant's version with amendments given above.	
Remarks	-	

Section 7.5.1.3 Terrestrial plant toxicity

Annex Point IIIA XIII.3.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1992): D1063 - Determination of effects on seedling growth of six plant species. [redacted] SLI Report [redacted] SLI Study# [redacted], date: 1992-06-23, (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	BASF SE	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Internal method entitled "Protocol for Conduct of a Seedling Growth Toxicity Test (Protocol # 073090/SGR:FDA.PG) based on procedures described in the FDA Environmental Assessment Technical Assistance Document 4.07, March 1987.	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 METHOD	
3.1	**Test material**	[redacted]	
3.1.1	Lot/Batch number	non-radioabelled TS) Lot No. [redacted] radiolabelled TS) Batch No [redacted]	
3.1.2	Specification	non-radioabelled TS) As given in section 2 of dossier radiolabelled TS) Specific activity: 12.7 µCl/mL	x
3.1.3	Purity	non-radioabelled TS) 99.7% radiolabelled TS) 98%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	A portion of each sample is combusted and counted by liquid scintillation counting (LSC) following laboratory SOP. Supplemental analyses were conducted to confirm the concentration of parent in the treated sand. The samples were extracted with acetone and analysed for parent D[redacted] by Rad-HPLC.	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII.3.4

3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	For each test concentration, the appropriate quantities of radiolabelled and unlabelled test substances are weighed out and dissolved in acetone, then mixed with the total volume of sand in a mechanical mixer for five minutes. The same volume of acetone is used for each test concentration and the solvent control. The sand is distributed in a tray to facilitate evaporation of the acetone then placed in a dark ventilated area. The sand is manually turned over at least twice per day for two days. Within one day of seedling, three distinct samples of the sand for each test substance concentration are collected.	
3.3	**Reference substance**	Unlabelled triclosan	
3.3.1	Method of analysis for reference substance		
3.4	**Testing procedure**		
3.4.1	Dilution water	-	
3.4.2	Test plants	Six species were tested: three monocotyledons, corn (*Zea mays*), ryegrass (*Lolium perenne*), and wheat (*Triticum aestivum*), and three dicotyledons, cucumber (*Cucumis sativus*), soybean (*Glycine max*), and tomato (*Lycopersicon esculentum*). See table A7_5_1_3-2	x
3.4.3	Test system	See table A7_5_1_3-3	
3.4.4	Test conditions	- For each species, five seedlings were planted in each of five replicate pots per concentration (containing quartz sand and placed in a saucer with a nutrient medium). See table A7_5_1_3-4	x
3.4.5	Test duration	21 days	
3.4.6	Test parameter	Morphological abnormalities (e.g., wilt, necrosis, chlorosis, foliar lesions and leaf blotch) and mortalities were recorded daily for each plant. Seedling shoot lengths were measured to establish growth rate curves. Light intensity, carbon dioxide and relative humidity were monitored daily in the growth chamber.	
3.4.7	Sampling	Seedling shoot lengths were measured on days 0, 1, 3, 5, 7, 14, and 21 to establish growth rate curves. Morphological abnormalities (e.g. wilt, necrosis, chlorosis, foliar lesions, and leaf blotch) and mortalities were recorded daily. At the conclusion of the 21-day exposure period, all surviving seedlings were severed at the soil surface and measured; roots were removed and washed to remove the sand and together with the shoots were dried and weighed.	
3.4.8	Method of analysis of the plant material	A plant was determined to be dead if it lost all natural green colour, which was generally accompanied by dehydration and loss of leaves. The shoot and roots of dead plants were either carefully removed from the support medium or the shoots were cut approximately 1 cm above the sand surface and the roots were left in the pot until test termination. At the conclusion of the 21 day exposure period, all surviving seedlings were severed at the soil surface and measured. Roots were gently removed and washed with deionised water to remove the sand.	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII.3.4

		Shoot length measurements were made from the base of the plants to the apical bud. Shoot and root material were dried for several days at 70°C at radiant heat ovens (Blue M 1.9 KW and Labline Imperial II) After drying, each was weighed to the nearest 0.1 mg (Scientific Products balance, model #182).	
3.4.9	Quality control	Light intensity, relative humidity, and CO_2 were recorded continuously from inside the chamber.	
3.4.10	Statistics	Percentage survival data were analyzed by Fisher´s Exact Test. Replicate mean values for shoot length, shoot weight, which were calculated from individual observations, were used during the statistical analyses. Length and weight were first tested for normality using the Chi-Square test and for homogeneity of variance using Bartlett´s test (Horning and Weber, 1985). Data sets that did not initially pass Bartlett´s test were transformed (1/Y inverse) and reanalysed using these tests. If the data set met these assumptions for parametric statistics, then one-way analysis of variance (Sokai and Rohlf, 1981) and a two-tailed Dunett´s test (Dunett, 1955, 1964) were performed to determine which treatment group were significantly different from the solvent control data. All statistical conclusions were made at the 95% level certainty ($P \leq 0.05$). These statistical procedures, used to determine statistically significant reduction effect, define the LOEC and NOEC for each species. Regression analyses were also performed on all shoot length, shoot weight and root weight data, to test for a linear effect of concentration. **Horning, W. B. and Weber, C. I. (1985): Short term methods for estimating the chronic toxicity of effluents and receiving waters to freshwaters organisms. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cicinnati, Ohio. EPA/600/4-85/014.** **Sokai, R. R. and Rohlf, F. J. (1981): Biometry. W. H. Freeman and Company, San Francisco.** **Dunett, C. W. (1955): A multiple comparison procedure for comparing several treatments with a control. J.Amer. Stat. Assoc. 50:1096-1121.** **Dunett, C. W. (1964): New tables for multiple comparisons with a control. Biometrics 20: 482-491.**	x
		4 RESULTS	
4.1	**Results test substance**	See table A7_5_1_3-5 and table A7_5_1_3-6 for results	
4.1.1	Applied initial concentration	Seedlings of each species were exposed to nominal concentrations of 10, 30, 100, 300, and 1000 µg a.s./kg as well as solvent (acetone) and blank controls. The test solutions were prepared in acetone and mixed into the quartz sand immediately prior to transplanting the seedlings.	
4.1.2	Phytotoxicity rating	At test termination, the morphological abnormalities observed (chlorosis, brown leaf tip, necrosis and foliar lesions) in corn, cucumber, ryegrass, tomato and wheat treatment plants did not appear to be related to the concentration of D1063, as compared to the control and solvent control plants. At termination of the soybean test, the presence of foliar lesions on plants exposed to the higher concentrations of the test material did appear to be concentration related. In general results of Fisher´s Exact test demonstrated that survival was not significantly affected by the exposure to D1063.	x x

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII.3.4

4.1.3	Shoot length	No statistically significant concentration-related reductions were observed in mean shoot length of the plants in any test concentration as compared to the solvent control plants. For cucumber the NOEC was determined to be 96 µg a.i./kg for the most sensitive parameter, i.e., shoot length.	x
4.1.4	Shoot dry weights	No statistically significant concentration-related reductions were observed in mean shoot length of the plants in any test concentration as compared to the solvent control plants. For tomato and wheat the NOEC was determined to be 280 µg a.i./kg for the most sensitive parameter, i.e., shoot dry weight.	x x x
4.1.5	Root dry weights		x
4.1.6	Root length	Not measured	
4.1.7	Number of dead plants	-	
4.1.8	Emergence data	-	
4.1.9	Concentration / response curve	Regression analysis performed ($P \leq 0.05$) on shoot length, shoot weight and root weight demonstrated that linear effects of concentration were observed on at least one of these parameters for all species except corn.	
4.1.10	Other effects	-	
4.2	**Results of controls**		
4.2.1	Number/ percentage of plants showing adverse effects	-	
4.2.2	Nature of adverse effects	-	
4.3	**Test with reference substance**	Not performed	
4.3.1	Concentrations		
4.3.2	Results		
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	A study was conducted to determine the potential effects of D[redacted]on seedling growth and survival of six plant species (corn, cucumber, ryegrass, soybean, tomato and wheat) over 21 days according to procedures described in the FDA Environmental Assessment Technical Assistance Documents 4.07, March 1987. Twenty-five seedlings per species (5 replicates, each containing 5 plants) were exposed to five concentrations of[redacted]a solvent control and a control. Each species were exposed to nominal test concentrations of 1000, 300, 100, 30 and 10 µg A.I./kg. Supplemental analyses were conducted to confirm the concentration of parent in the treated sand. At the initiation and termination of the study sand samples were extracted with acetone and analyzed for parent	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII.3.4

		[redacted] by RAD-HPLC.	
5.2	**Results and discussion**	At the initiation of the study, 89-97% of the radioactivity extracted into acetone and chromatographed as the test substance. At the termination of the study, no parent, [redacted] was recovered from two samples. In the remaining seven samples, only 10-34 % of the radioactivity initially added was recovered as parent. At test termination, the morphological abnormalities observed (chlorosis, brown leaf tip, necrosis and foliar lesions) in corn, cucumber, ryegrass, tomato and wheat treatment plants did not appear to be related to the concentration of [redacted] as compared to the control and solvent control plants. At termination of the soybean test, the presence of foliar lesions on plants exposed to the higher concentrations of the test material did appear to be concentration related. In the vegetative vigour study, the following results were obtained with the six species tested: - Corn, soybean: no treatment-related effects (NOEC > 930 µg/kg) - Cucumber: most sensitive parameter was shoot length (NOEC = 280 µg/kg) - Ryegrass: most sensitive parameter was root weight (NOEC = 930 µg/kg) - Wheat: most sensitive parameter was shoot weight (NOEC = 930 µg/kg) - Tomato: most sensitive parameter was root/shoot weight (NOEC = 930 µg/kg)	x
5.2.1	EC_0	Not applicable	
5.2.2	EC_{50}	Not applicable	
5.2.3	EC_{80}	Not applicable	
5.3	**Conclusion**	Corn and soybean were determined to be least sensitive species to the toxicity of D[redacted]B, each with a NOEC of 930 µg A.I./kg. Ryegrass, tomato and wheat were more sensitive to the exposure, each having a NOEC of 280 µg A.I./kg. Cucumber was determined to be the most sensitive species with a NOEC of 96 µg A.I./kg. No stimulatory growth effects of [redacted] were observed for any species.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section 7.5.1.3 Terrestrial plant toxicity
Annex Point IIIA XIII.3.4

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	April 2012
Materials and Methods	3.1.2. Statement regarding Section 2 not applicable for DCPP. 3.4.2: Table A7_5_1_3-2: Move wheat from Dicotyledone box to Moncotyledone box 3.4.2: Table A7_5_1_3-4: correct temperature from 30 to 27°C. 3.4.10: *Insert* "and root weight" after term "shoot weight".
Results and discussion	4.1.2: *Deletion:* "cucumber" 4.1.2: *Addition:* "Foliar lesions were observed in several of the treatment plants at the three highest treatment levels for cucumber". 4.1.3: *Addition:* "for corn, ryegrass, wheat, soybean and tomato" after term "mean shoot length of the" 4.1.4: *Substitution:* Exchange "mean shoot length" by "mean shoot dry weight". 4.1.4: *Addition:* "for corn, ryegrass and soybean" after term "mean shoot dry weight" 4.1.4: *Addition:* In cucumber plants a statistically significant concentration-related reduction in mean shoot weight was observed at the highest treatment concentration (930 µg a.i./kg). 4.1.5: *Addition:* "No statistically significant concentration-related reductions were observed in mean root dry weight of the corn, soybean and wheat plants in any test concentration as compared to the solvent control plants. For ryegrass and tomato the NOEC was determined to be 280 µg a.i./kg for the most sensitive parameter, i.e., root dry weight. In cucumber plants a statistically significant concentration-related reduction in mean root weight was observed at the highest treatment concentration (930 µg a.i./kg).
Conclusion	5.2: *Addition:* "Foliar lesions were observed in several of the treatment plants at the three highest treatment levels for cucumber".
Reliability	2
Acceptability	Acceptable for environmental risk assessment.
Remarks	-

Table A7_5_1_3-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes, acetone
Concentration of vehicle	Not stated
Vehicle control performed	Yes
Other procedures	-

Table A7_5_1_3-2: Test plants

	Family	Species	Common name	Source (seed/plant)
Monocotyledonae		*Zea mays*	Corn	[redacted]
		Lolium perenne	Ryegrass	[redacted]
Dicotyledonae		*Glycine max*	Soybean	[redacted]
		Lycopersicon esculentum	Tomato	[redacted]
		Triticum aestivum	Wheat	[redacted]
		Cucumis sativus	Cucumber	[redacted]

Table A7_5_1_3-3: Test system

Criteria	Details
Test type	Growth chamber with controlled environmental conditions
Container type	13 cm tall (13 cm top diameter and 9 cm bottom diameter) polypropylene pot (Kord Products Limited.
Seed germination potential	-
Identification of the plant species	All pots were labelled with the study No. and sample code
Number of replicates	5
Numbers of plants per replicate per dose	5 seeds
Date of planting	-
Plant density	Five plants per replicate
Date of test substance application	-
Heigh of plants at application	1-3 cm tall after planting
Date of phytotoxicity rating or harvest	Morphological abnormalities were observed daily and mortalied wer recorded daily. Shoot length measurements were made on Day 0, 1, 3, 5, 7, 14 and 21. Dried shoot weights, and dried root weights were determined after test termination (after 21 days).
Dates of analysis	Day 0, 1, 3, 5, 7, 14, 21

Table A7_5_1_3-4: Test conditions

Criteria	Details
Test type	Terrestrial plants, seed germination and growth test according to a Seedling Growth Toxicity Test
Method of application	The active substance was mixed into the sand
Application levels	-
Dose rates	10, 30, 100, 300, and 1000 µg test item/kilogram of dry weight soil
Substrate characteristics	sand
Watering of the plants	A nutrient solution containing necessary minerals and minor elements for plant growth was continuously provided to seedlings by subirrigation.
Temperature	20 - 30 °C.
Thermoperiod	-
Light regime	Artificial lighting (combination of commercial fluorescent and 34 watt incandescent bulbs (Duro-Test Vita-Lite HO 800 MA fluorescent bulbs and Duro-Test Wattsaver incandescent bulbs). 16-h-light / 8-h-dark cycle
Relative humidity	≥ 60%
Wind volatility	-
Observation periods and duration of test	Duration: 21 days
Pest control	No
Any other treatments and procedures	-

Table A7_5_1_3-5: Effective phytotoxicity

Mean measured conc.	Shoot length (cm)		Percent survival	Dry weight (g)	
µg a.i./kg	Day 0	Day 21	Day 21	Root	Shoot
Corn					
930	1.9	66.9	96	0.1830	0.4938
280	1.6	74.5	100	0.2228	0.5951
96	1.6	68.6	100	0.1853	0.4629
33	1.4	75.9	100	0.1854	0.5547
11	1.6	70.7	80	0.1576	0.5156
Solvent control	1.7	72.3	100	0.1831	0.5159
Control	1.7	67.5	100	0.2070	0.4960
Cucumber					
930	1.2	14.3	96	0.0509	0.2774
280	1.1	21.9	100	0.1318	0.6200
96	1.2	25.0	100	0.1238	0.6932
33	1.1	27.5	100	0.1207	0.7877
11	1.2	25.8	100	0.1218	0.7187
Solvent control	1.2	28.1	100	0.1472	0.7541
Control	1.2	26.5	100	0.1055	0.7534
Ryegrass					
930	2.1	17.4	100	0.0085	0.0246
280	1.9	17.1	100	0.0152	0.0269
96	1.8	22.6	88	0.0156	0.0414
33	1.8	24.5	100	0.0210	0.0580
11	1.8	20.7	100	0.0147	0.0371
Solvent control	2.0	20.4	92	0.0237	0.0376
Control	2.0	22.1	96	0.0212	0.0432
Soybean					
930	1.0	48.0	96	0.0823	0.4792
280	1.0	49.7	100	0.0919	0.5825
96	1.0	45.3	88	0.0778	0.5363
33	1.0	51.8	96	0.0974	0.6562
11	1.0	55.0	96	0.1029	0.6751
Solvent control	1.0	53.8	96	0.0878	0.5837
Control	1.1	61.9	100	0.1119	0.7443

Table A7_5_1_3-5: Effective phytotoxicity (cont.)

Mean measured conc.	Shoot length (cm)		Percent survival	Dry weight (g)	
µg a.i./kg	Day 0	Day 21	Day 21	Root	Shoot
Tomato					
930	1.3	5.6	96	0.0138	0.0462
280	1.2	6.7	96	0.0173	0.0607
96	1.2	6.3	100	0.0182	0.0675
33	1.3	8.4	100	0.0295	0.1058
11	1.1	7.5	100	0.0232	0.0978
Solvent control	1.2	7.2	96	0.0298	0.0909
Control	1.3	8.6	100	0.0525	0.1693
Wheat					
930	2.3	25.0	100	0.0446	0.0697
280	2.1	26.4	96	0.0478	0.0831
96	2.3	26.0	92	0.0486	0.0779
33	2.1	28.4	88	0.0592	0.1051
11	2.1	26.4	96	0.0429	0.0833
Solvent control	2.1	27.6	96	0.0555	0.0978
Control	2.0	27.9	96	0.0559	0.0948

Table A7_5_1_3-6: LOEC and NOEC results for each species

Table 10. Lowest observed effect concentrations (LOEC) and no observed effect concentrations (NOEC) for each species, based upon initial measured concentrations, in µg A.I./kg.

Species	LOEC (µg A.I./kg)[a]	Most Sensitive Parameter(s)	NOEC (µg A.I./kg)[a]
Corn	>930	No effect	930
Cucumber	280	Shoot length	96
Ryegrass	930	Root weight	280
Soybean	>930	No effect	930
Tomato	930	Root weight and shoot weight	280
Wheat	930	Shoot weight	280

[a] The LOEC and NOEC values were determined by the most sensitive parameter, i.e., shoot length, shoot weight, root weight or survival, according to Dunnett's Test or Fisher's Exact Test.

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

Official use only

Experimental data on the chronic effects of Triclosan to the earthworm are used to assess the ecotoxicological effect of DCPP.

The proposed source chemical (Triclosan) has the same basic structure as the target chemical (DCPP) but contains an additional chlorine atom on the ring without the hydroxyl group. Based on a comparison of physico-chemical and ecotoxicological data on DCPP and Triclosan, it can be concluded that both substances have similar physic-chemical properties. The toxicity of Triclosan is higher than that of DCPP. Therefore, the data on Triclosan can be used to fill data gaps for DCPP. This approach can be regarded as a worst-case approach. For a detailed assessment of this read-across approach see the separate document "Justification of the analogue approach".

1.1 Reference	[redacted](2010) Effects of [redacted](Triclosan) on Survival, Growth, and Reproduction of the Earthworm *Eisenia fetida*. [redacted] Laboratories Study report Nr. [redacted] 09-Apr-2010
1.2 Data protection	Yes
1.2.1 Data owner	BASF SE
1.2.2 Companies with letter of access	Not applicable since submitter is data owner
1.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study	Yes, OECD TG 222: Earthworm Reproduction Test (*Eisenia fetida / Eisenia andrei*), April 2004
2.2 GLP	Yes (with Certificate)
2.3 Deviations	No

3 METHOD

3.1 Test material	[redacted](Triclosan)
3.1.1 Lot/Batch number	[redacted]
3.1.2 Specification	As given in section 2
3.1.3 Purity	100.4%
3.1.4 Composition of Product	See purity
3.1.5 Further relevant properties	White solid powder, stored at room temperature at about 20 °C, away from direct sunlight; solubility in water: 0.012 g/L at 20 °C at pH 7 0.0065 g/L at 20 °C at pH 5
3.1.6 Method of analysis	The analytical measurement of the test item was based on HPLC/MS following extraction with acetone/hexane
3.2 Reference substance	Yes, Derosal® (a.i. Carbendazim, 60%)

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

	0	READ-ACROSS JUSTIFICATION
3.3	Testing procedure	

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

3.3.1 Preparation of the test substance	No aqueous application solution could be prepared for the dosing of the test item due to its limited water solubility. However, the test item was completely soluble in acetone at the required concentrations. Thus, the test item was dissolved and diluted in acetone and applied to a portion of sand. An application solution for the highest test item concentration was prepared by dissolving 1250.1 mg of the test item completely in 50 mL of acetone, resulting in a test item concentration of 25 mg/mL acetone. This application solution was serially diluted with acetone to obtain application solutions for the lower test concentrations scheduled.
3.3.2 Application of the test substance	For each test concentration, 12 mL of the corresponding application solution was applied to 60 g sand in a mortar. The solvent was completely evaporated at room temperature under a hood in 2 hours. The remainder (sand/test item mixture) was intensively mixed by means of a pestle and then mixed on a roller mixer for 30 minutes. Then, the sand/test item mixture was divided into aliquots of 10 g (corrected for the amount of test item applied to 10 g of sand) and filled into separate vials. The individual mixtures were prepared before test start. Immediately before test start, aliquots of 10 g sand/test item mixture were quantitatively incorporated into the four replicates of artificial soil per test concentration and the moisture content was adjusted
3.3.3 Reference substance	A single concentration of 8.33 mg Derosal®/kg dry soil (corresponding to 5.0 mg a.i./kg dry soil) was tested. The application solution for the item Derosal® was prepared by mixing 41.7 mg test item homogeneously into 1000 mL purified water. Then, 100-mL aliquots of this application solution were incorporated into the artificial soil of each replicate (500 g dry weight per replicate) by intense mixing in a laboratory mixer and the moisture content was adjusted. In the reference treatment with Derosal®, the same artificial soil as for Triclosan was used.
3.3.4 Test organisms	For details on tested organisms see table A7_5_1_1-1
3.3.5 Test system	For details on test type, laboratory equipment, test substrate, replicates etc. see table A7_5_1_1-2
3.3.6 Test conditions	For details on relevant test conditions see table A7_5_1_1-3
3.3.7 Test duration	8 weeks
3.3.8 Test parameter	Mortality, adverse effects, body weight, food consumption, reproduction
3.3.9 Examination	Mortality, adverse effects: After four weeks of exposure, the content of each test vessel was emptied and the living adult worms were counted and checked for any abnormal behavior or other adverse effects (worms not moving after gentle mechanical stimulus to their front end are considered to be dead, also missing worms are considered to have died since dead worms can completely decompose in the soil in short time and hence cannot always be found). Body weight: At the beginning (prior to exposure) and end of the first four weeks of the test, the adult test organisms of each test vessel were weighed (at the start each individually, at the end all together in each test vessel). Before weighing, the worms were quickly washed with water and surplus water was adsorbed on filter paper. For each test vessel, the difference of the mean body wet weight of the surviving test organisms between start and end of the exposure period was calculated. The changes in mean body weight of the surviving worms were compared to the control.

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

Food consumption:
At each feeding date, the amount of food consumed by the adult worms was visually estimated for each test vessel.
Reproduction:
At the test termination after 8 weeks, the number of living juveniles per test vessel was determined. The test vessels were warmed up in a water bath at 60 °C for approximately 15-20 minutes to encourage the living juvenile earthworms to rise to the soil surface. The juvenile earthworms at the soil surface were counted and removed. The test vessels were warmed up for a second time for about 10 minutes and the surface was searched for juveniles. Afterwards, each test vessel was emptied and the soil was intensely searched for eventually remaining juveniles. The reproduction rates of the surviving test organisms at the end of the test were compared to the control.

3.3.10 Monitoring of test substance concentration: Yes, day 0, day 14, day 28, day 56

3.3.11 Statistics: The changes in mean body weight of surviving worms compared to the control were statistically evaluated by means of a multiple Dunnett-t test (Dunnett CW (1955) Journal of the American Statistical Association 50: 1096-1121; Dunnett CW (1964) Biometrics 20: 482−491) after a one-way analysis of variance (ANOVA).
The body weight change of the surviving worms in the reference item treatment was compared to the control and was statistically evaluated by means of a Student-t test (α=0.05, one-sided smaller; Sachs l (1999) Angewandte Statistik, 9th Edition, Springer Verlag, Berlin).
The reproduction rates of the surviving test organisms at the end of the test were compared to the control values and were statistically evaluated by means of a multiple Dunnett-t test after a one-way analysis of variance (ANOVA). The reproduction in the reference item treatment was compared to the control and was statistically evaluated by means of a Mann-Whitney-U test (α=0.05, one-sided smaller).

4 RESULTS

4.1 Analytical monitoring of test concentrations: Performed

4.1.1 Summary of analytical results

Nominal test conc. (mg/kg dry soil)	Measured concentration of Triclosan (mg/kg dry soil) #									
	at Day 0		at Day 14		at Day 28		at Day 56		at Day 0-56	
		% of nominal		% of nominal		% of nomina		% of nominal	geometric mean	% of nominal
control	<0.05	---	<0.05	---	<0.05	---	<0.05	---	<0.05	---
0.32	0.32	100.0	0.28	87.5	0.25	78.1	0.26	80.0	0.28	86.0
1.0	0.98	98.0	0.89	89.0	0.81	81.1	0.74	73.8	0.85	85.0
3.2	3.20	100.0	2.70	84.4	2.38	74.4	2.24	70.0	2.61	81.4
10	9.70	97.0	8.70	87.0	8.00	80.0	7.53	75.3	8.44	84.4
32	32.80	102.5	31.50	98.4	28.90	90.3	26.90	84.1	29.94	93.6
100	114.00	114.0	101.00	101.0	95.00	95.0	98.00	98.0	101.75	101.8

#: geometric mean of the measured test item concentration taken from Sponsor's analytical report (study number for correspondence: 09.260).

4.1.2 Measured versus nominal concentrations: The mean measured concentrations were about 81 to 102% of the nominal values.

4.2 Toxicity findings

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)
Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.2.1 Mortality

In the control group and at all other treatments, no mortality was determined.

4.2.2 Adverse effects

No adverse effects were observed in the adult test organisms at any test concentration.

4.2.3 Body weight

4.2.3.1 Mean body wet weights of adult earthworms at the test start and after 4 weeks of exposure

Nominal/Mean measured test item conc. (mg/kg dry soil)[a]	Vessel No.	Test start (Day 0): Weight of all worms (mg)	Test start (Day 0): No. of worms	Test start (Day 0): Mean body weight/worm (mg)	After 4 weeks (Day 28): Weight of all worms (mg)	After 4 weeks (Day 28): No. of worms	After 4 weeks (Day 28): Mean body weight/worm (mg)	Difference to test start: Mean difference per worm (mg)	Difference to test start: Mean difference per worm (%)	Difference to test start: stat.[a]
Control	1	4181	10	418	3957	10	396	-22	-5%	
	2	4286	10	429	4226	10	423	-6	-1%	
	3	4286	10	429	4787	10	479	50	12%	
	4	4115	10	412	4098	10	410	-2	0%	
	5	4548	10	455	4608	10	461	6	1%	
	6	4405	10	441	4229	10	423	-18	-4%	
	7	4315	10	432	4588	10	459	27	6%	
	8	4725	10	473	4728	10	473	0	0%	
	Mean:			436			440	4	1%	—
	SD:			20			31	24		
0.32 / 0.28	1	4337	10	434	4329	10	433	-1	0%	
	2	4261	10	426	4132	10	413	-13	-3%	
	3	4270	10	427	4309	10	431	4	1%	
	4	4322	10	432	4610	10	461	29	7%	
	Mean:			430			435	5	1%	n.s.
	SD:			4			20	18		
1.0 / 0.85	1	4479	10	448	4526	10	453	5	1%	
	2	4368	10	437	4311	10	431	-6	-1%	
	3	4051	10	405	4303	10	430	25	6%	
	4	3767	10	377	4163	10	416	40	11%	
	Mean:			417			433	16	4%	n.s.
	SD:			32			15	20		
3.2 / 2.61	1	4574	10	457	4557	10	456	-2	0%	
	2	4322	10	432	4459	10	446	14	3%	
	3	4017	10	402	4268	10	427	25	6%	
	4	4033	10	403	4411	10	441	38	9%	
	Mean:			424			442	19	5%	n.s.
	SD:			27			12	17		
10 / 8.44	1	4417	10	442	4195	10	420	-22	-5%	
	2	4603	10	460	4582	10	458	-2	0%	
	3	4380	10	438	4496	10	450	12	3%	
	4	4453	10	445	4663	10	466	21	5%	
	Mean:			446			448	2	0%	n.s.
	SD:			10			20	19		
32 / 29.94	1	4055	10	406	4443	10	444	39	10%	
	2	4500	10	450	4673	10	467	17	4%	
	3	4121	10	412	4529	10	453	41	10%	
	4	4563	10	456	4871	10	487	31	7%	
	Mean:			431			463	32	8%	n.s.
	SD:			26			19	11		
100 / 101.75	1	4307	10	431	4611	10	461	30	7%	
	2	4555	10	456	4811	10	481	26	6%	
	3	4241	10	424	4751	10	475	51	12%	
	4	4151	10	415	4622	10	462	47	11%	
	Mean:			431			470	39	9%	n.s.
	SD:			17			10	12		
Derosal® 8.33 mg/kg dry soil	1	4342	10	434	3807	10	381	-54	-12%	
	2	4287	10	429	4002	10	400	-29	-7%	
	3	4040	10	404	4120	10	412	8	2%	
	4	4116	10	412	4222	10	422	11	3%	
	Mean:			420			404	-16	-4%	n.s.[b]
	SD:			14			18	31		

Note: Calculations were performed with a commercial computer program (EXCEL). The results given in the table are rounded values.
a: Statistical comparison of the changes in mean body wet weight of the treatments with the control (absolute mean differences from test start to test end):
n.s.: mean value not statistically significantly different compared to the control (Dunnett-t test, one-sided smaller, α=0.05).
#: geometric mean of the measured test item concentration taken from Sponsor's analytical report (study number for correspondence: 09.260).

4.2.3.2 Effect of the treatment on body weight

The mean body weight of the adult worms in the control had increased during the 4-week exposure period on average by 4 mg per worm (1% of the mean initial weight). At all test concentrations of Triclosan, the increase in the mean body weight of the adult worms was almost the same or even slightly higher than in the control, and thus, it was not statistically significantly lower than in the control.

4.2.4 Food consumption

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.2.4.1 Feeding and food consumption during the test period

Nominal/Mean measured test item conc. (mg/kg dry soil) *	Vessel No.	Food added Day 1 (g)	Day 7 (g)	Day 14 (g)	Day 21 (g)	Day 28 * (g)	Sum (g)
Control	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
	5	6	4	4	4	6	24
	6	6	4	4	4	6	24
	7	6	4	4	4	6	24
	8	6	4	4	4	6	24
0.32 / 0.28	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
1.0 / 0.85	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
3.2 / 2.61	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
10 / 8.44	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
32 / 29.94	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
100 / 101.75	1	6	4	4	4	6	24
	2	6	4	4	4	6	24
	3	6	4	4	4	6	24
	4	6	4	4	4	6	24
Derosal® 8.33 mg/kg dry soil	1	6	2 a	2 a	2 a	6	18
	2	6	2 a	2 a	2 a	6	18
	3	6	2 a	2 a	2 a	6	18
	4	6	2 a	2 a	2 a	6	18

*: Food mixed into the test substrate after removal of the adults on Day 28.
a: Food partly consumed during the previous week, some food had remained on the surface of the test substrate.

4.2.4.2 Effect of the treatment on food consumption

No difference in food consumption was determined between the control and all test item concentrations.

4.3 Reproduction

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.3.1 Total number of living offspring produced by all adults within 28 days of continuous exposure, expressed per test vessel and per surviving adult

Nominal/Mean measured test item conc. (mg/kg dry soil)*	Vessel No.	Surviving adults	Juvenile worms: per vessel No.	mean	SD	CV (%)	per surviving adult No.	mean	SD	CV (%)	% of control	Statistical evaluation*
Control	1	10	149				14.9					
	2	10	112				11.2					
	3	10	91				9.1					
	4	10	109				10.9					
	5	10	109				10.9					
	6	10	109				10.9					
	7	10	138				13.8					
	8	10	98	114	19.5	17.1	9.8	11.4	2.0	17.1	100.0	---
0.32 / 0.28	1	10	98				9.8					
	2	10	140				14.0					
	3	10	102				10.2					
	4	10	91	108	22.0	20.4	9.1	10.8	2.2	20.4	94.2	n.s.
1.0 / 0.85	1	10	113				11.3					
	2	10	156				15.6					
	3	10	114				11.4					
	4	10	94	119	26.2	21.9	9.4	11.9	2.6	21.9	104.3	n.s.
3.2 / 2.61	1	10	141				14.1					
	2	10	115				11.5					
	3	10	102				10.2					
	4	10	97	114	19.7	17.3	9.7	11.4	2.0	17.3	99.5	n.s.
10 / 8.44	1	10	102				10.2					
	2	10	143				14.3					
	3	10	129				12.9					
	4	10	113	122	18.0	14.8	11.3	12.2	1.8	14.8	106.4	n.s.
32 / 29.94	1	10	99				9.9					
	2	10	126				12.6					
	3	10	127				12.7					
	4	10	96	112	16.8	15.0	9.6	11.2	1.7	15.0	97.9	n.s.
100 / 101.75	1	10	132				13.2					
	2	10	106				10.6					
	3	10	142				14.2					
	4	10	112	123	16.9	13.7	11.2	12.3	1.7	13.7	107.5	n.s.
Derosal® 8.33mg/kg dry soil	1	10	0				0.0					
	2	10	0				0.0					
	3	10	0				0.0					
	4	10	0	0	0.0		0.0	0.0	0.0		0.0	s.*

Note: Calculations were performed with a commercial computer program (EXCEL). The results given are rounded values.
* Statistical comparison of the changes in mean body wet weight of the treatments with the control (absolute mean differences from test start to test end):
n.s.: mean value not statistically significantly different compared to the control (Dunnett-t test, one-sided smaller, α=0.05).
s.*: mean value statistically significantly smaller than the control (Mann-Whitney-U test, one-sided smaller, α=0.05).
#: geometric mean of the measured test item concentration taken from Sponsor's analytical report (study number for correspondence: 09.260).

4.3.2 Reproduction rate

In the control, an average of 114 juvenile worms per test vessel were found (corresponding to a mean reproduction rate of 11.4 juveniles per surviving adult). The coefficient of variance of the reproduction rate per test vessel and per surviving individual in the control was 17.1%. Thus, the validity criteria of the test guidelines (at least 30 juveniles per test vessel, coefficient of variance of reproduction ≤30%) were well fulfilled. At all test item concentrations, the mean reproduction rates per surviving adult worm corresponded to 94-108% of the control value. The mean reproduction rates were not statistically significantly reduced up to and including the highest test item concentration of 100 mg/kg dry soil Triclosan.

4.4 NOEC/LOEC, EC50

4.4.1 NOEC

Taking into account the survival, growth and reproduction rates of the exposed adult test organisms, the highest nominal concentration of Triclosan of 100 mg/kg dry soil (mean measured 101.8 mg/kg dry soil) was set as NOEC for *Eisenia fetida* in the Earthworm Reproduction Test according to OECD TG 222 (2004).

4.4.2 EC_{50}

The EC_{50} value for reproduction could not be calculated due to the absence of a toxic effect of Triclosan on the reproduction rate *of Eisenia fetida* and was determined to be > 100 mg/kg dry soil.

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.5	**Test with reference substance**	Performed
4.5.1	Concentrations	A single concentration of the reference item Derosal® of 8.33 mg/kg formulation corresponding to 5 mg Carbendazim as a.i./kg was tested.
4.5.2	Results	At a single concentration of 8.33 mg/kg of Derosal® (i.e., 5 mg a.i./kg), no mortality of the adult worms was determined. A mean decrease of 4% in body wet weight of the earthworms in the positive control during the exposure period was reported, which was not statistically significantly different to the control. The reproduction rate of *Eisenia fetida* in the positive control was completely inhibited and thus statistically significantly reduced in comparison to the control. For details see table in 4.3.1. Thus, the results of the positive control demonstrated satisfactory test conditions.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxic effects of [redacted](Triclosan) on survival, growth and reproduction of the earthworm *Eisenia fetida* were assessed during a test period of eight weeks based on the OECD Guidelines for Testing Chemicals, Test No. 222 (April 2004); the study conduct was GLP conform. Test substance: Triclosan, Batch Nr. [redacted] purity 100.4% Test system: *Eisenia fetida*, 5–6 months old adults with a clitellum, individual body wet weight ranged from 308 to 598 mg The test item was homogeneously mixed into artificial soil at the following nominal test concentrations: 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg dry soil. The analytical monitoring of the test concentration revealed mean measured test item concentrations of 0.28, 0.85, 2.61, 8.44, 29.94 and 101.75 mg/kg dry soil, respectively; thus, the mean measured concentrations ranged between 80 and 102 % of the nominal values. Additionally, a negative control and a reference item treatment with 8.33 mg Derosal®/kg dry soil (corresponding to 5.0 mg Carbendazim as a.i./kg dry soil) were tested in parallel. The earthworms were exposed in treated soils for a period of four weeks. After this period, the adults were removed from the test vessels and the survival and growth rate were determined. The cocoons and juvenile earthworms remained in the vessels for additional four weeks. The reproduction rate was determined by counting the number of offspring hatched from the cocoons after this additional test period of four weeks.
5.2	**Results and discussion**	Toxicity: In the control group and at all other treatments, neither mortality nor adverse effects was observed. The mean body weight of the adult worms in the control had increased during the 4-week exposure period on average by 4 mg per worm (1% of the mean initial weight). At all test concentrations of Triclosan, the increase in the mean body weight of the adult worms was almost the same or even slightly higher than in the control, and thus, it was not statistically significantly lower than in the control. No difference in food consumption was determined between the control and all test item concentrations. At the single concentration of 8.33 mg/kg formulation, the reference item Derosal® no mortality or adverse effect of the adult worms was determined; a mean decrease of 4% in body wet weight of the earthworms was reported, which however was not statistically

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

		significantly different to the control. Impairment of reproduction: In the control, an average of 114 juvenile worms per test vessel were found (corresponding to a mean reproduction rate of 11.4 juveniles per surviving adult). The coefficient of variance of the reproduction rate per test vessel and per surviving individual in the control was 17.1%. Thus, the validity criteria of the test guidelines (at least 30 juveniles per test vessel, coefficient of variance of reproduction ≤30%) were well fulfilled. At all test item concentrations, the mean reproduction rates per surviving adult worm corresponded to 94-108% of the control value. The mean reproduction rates were not statistically significantly reduced up to and including the highest nominal test item concentration of 100 mg/kg dry soil Triclosan. The reproduction rate of *Eisenia fetida* in the positive control was completely inhibited and thus, demonstrated satisfactory test conditions.
5.2.1	NOEC	100 mg/kg dry soil (measured 101.8 mg/kg dry soil)
5.2.2	EC_0	100 mg/kg dry soil (measured 101.8 mg/kg dry soil)
5.2.3	EC_{50}	> 100 mg/kg dry soil (measured 101.8 mg/kg dry soil)
5.3	**Conclusion**	Since no effects on survival, growth and reproduction rates of *Eisenia fetida* were observed at nominal test concentrations of Triclosan up to and including 100 mg/kg dry soil (i.e. 101.8 mg/kg soil measured) the NOEC was set at 100 mg/kg dry soil. The EC_{50} for reproduction could not be calculated due to the absence of a toxic effect on reproduction up to the highest nominal test concentration of 100 mg/kg dry soil (measured 101.8 mg/kg dry soil); thus, the EC_{50} was considered to be > 100 mg/kg dry soil.
5.3.1	Other Conclusions	Since an average of 114 juvenile worms per test vessel were found in the control group, corresponding to a mean reproduction rate of 11.4 juveniles per surviving adult, and since the coefficient of variance of the reproduction rate per test vessel and per surviving individual in the control was 17.1%, the validity criteria of the test guidelines (at least 30 juveniles per test vessel, coefficient of variance of reproduction ≤30%) were well fulfilled. Furthermore, the reproduction rate of *Eisenia fetida* in the positive control was completely inhibited, indicating satisfactory test conditions.
5.3.2	Reliability	The study was guideline-conform, the validity criteria were fulfilled, and the test conduct followed GLP (certificate included in the report), thus, a reliability of 1 was given.
5.3.3	Deficiencies	None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	July 2013
Materials and Methods	The applicant's version is acceptable, however, for determination of NOEC, the concentrations should be spaced by a factor not 2.0. For a combined approach for determination of both the NOEC and ECx eight treatment concentrations in a geometric series should be used. The concentrations should be spaced by a factor not exceeding 1.8 in this case.
Results and discussion	Adopt applicant's version for the NOEC value

Section A7.5.2.1 Earthworm Reproduction Test (OECD TG 222)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

Conclusion	NOEC = 100 mg/kg (dw)
Reliability	2
Acceptability	Acceptable for environmental risk assessment
Remarks	While the read across is supported, it has to be stated that DCPP is of similar and not lower toxicity. The approach is therefore a realistic but not a worst case approach.

Table A7_5_1_1-1: Test organisms

Criteria	Details
Species/strain	*Eisenia fetida*
Source of the initial stock	Synchronized culture maintained at [redacted]
Culturing techniques	During breeding and keeping, they were fed with suitable food (i.e. horse manure and potatoes)
Age/weight	The worms used were 5–6 months old adults with a clitellum. The individual body wet weight of the test organisms at the start of the test ranged from 308 to 598 mg
Pre-treatment	For two days prior to the test start, the test organisms were acclimatized to the artificial soil and test temperature

Table A7_5_1_1-2: Test system

Criteria	Details
Artificial soil test substrate	The artificial soil was prepared according to the guidelines with the following constituents (percentage distribution on dry weight basis): - Sphagnum peat: 10% (shredded, sieved through 2 mm sieve) - Kaolinite clay: 20% (containing 35.1% Al_2O_3 and approx. 85% Kaolinite) - Sand (Sihelco 36): 69% (Size analysis: 1% (0.25 mm), 8% (0.18 mm), 80% (0.125 mm), 10% (0.09 mm), 1% (<0.09 mm) - Calcium carbonate ($CaCO_3$): 0.5% (for the adjustment to pH 6.0 ± 0.5) - Food (dried organic manure): 1%
Test mixture	The air dry components of the artificial soil (without calcium carbonate and food) were intensively mixed in a batch of 40 kg (dry weight) in a laboratory mixer to prepare a substrate mixture with the composition of peat, kaolinite clay, and sand as mentioned above. For each test vessel, 500 g (dry weight) of the prepared artificial soil was thoroughly mixed with 10 g sand/test item mixture, 2.5 g calcium carbonate, 5 g food, and 188 mL purified water in a laboratory mixer. The amount of 695 g test substrate (corresponding to 500 g dry weight) was filled into each test vessel resulting in a soil layer of approx. 5-6 cm. With the addition of 188 mL purified water to the test substrate of each test vessel, the soil moisture content was adjusted to 39%, corresponding to approximately 60% of the total water holding capacity (WHC) of the artificial soil. The water holding capacity (WHC) had been determined in two samples of the artificial soil (according to ISO guideline 11268-2) and the moisture of the test substrate was adjusted. At the start of the test, the soil moisture was checked in samples from the treatments. The WHC amounted in average to 65.3 g water per 100 g dry soil.
Size, volume and material of test container	Plastic boxes (length about 16.5 cm, width about 12 cm, height about 6.5 cm, area about 200 cm^2) were used as test vessels. Each test vessel contained a defined amount of dry artificial soil, corresponding to a layer depth of approximately 5-6 cm. The test vessels were covered by transparent lids to prevent worms from escaping and to reduce evaporation during the test period. The lids were perforated to allow air exchange. The test vessels were labeled with the study number and all necessary additional information to ensure unique identification.
Amount of artificial soil (kg)/ container	695 g test substrate (corresponding to 500 g dry weight) per vessel
Nominal levels of test concentrations	0.32, 1.0, 3.2, 10, 32 and 100 mg/kg dry soil
Number of replicates/concentration	four test vessels (replicates)
Number of earthworms/test concentration	40 test organisms were tested per test item treatment
Number of earthworms/container	ten earthworms per test vessel were used
Light source	Not specified
Test performed in closed vessels due to significant volatility of test substrate	No

Table A7_5_1_1-3: Test conditions

Criteria	Details
Test temperature	During the entire test period, the room temperature was in the range of 19.4 to 21.7 °C, and was thus within the limits required by the guidelines (18-22 °C).
Moisture content	Water content and pH of the artificial soil at test start and end: Nominal test item concentration (mg/kg dry soil) / Mean measured test item concentration (mg/kg dry soil) / Start of the test: pH / Start of the test: Water content (%) / After 8 weeks: pH / After 8 weeks: Water content (%) Control: 5.8 / 38 / 5.7 / 42 0.32 / 0.28: 5.7 / 38 / 5.8 / 41 1.0 / 0.85: 5.6 / 38 / 5.8 / 42 3.2 / 2.61: 5.6 / 38 / 5.8 / 43 10 / 8.44: 5.7 / 38 / 5.9 / 42 32 / 29.94: 5.6 / 37 / 5.9 / 41 100 / 101.75: 5.6 / 38 / 5.9 / 43 Derosal® 8.33 mg/kg dry soil: 5.6 / 37 / 5.8 / 40 The soil moisture content was sufficiently constant (37-38% at test start and 41-43% at test termination after 8 weeks
pH	See table above. The pH value in the untreated artificial soil was 5.8 at the start of the test. At the test end, the pH in the test replicates was between 5.7 and 5.9.
Adjustment of pH	Yes, initially, by addition of 0.5% calcium carbonate ($CaCO_3$) to the artificial soil
Light intensity / photoperiod	Light intensity within 523 to 584 Lux 16-hour light to 8-hour darkness
Relevant degradation products	Not applicable

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

Official use only

Experimental data on the chronic effects of Triclosan to the predatory mite are used to assess the ecotoxicological effect of DCPP.

The proposed source chemical (Triclosan) has the same basic structure as the target chemical (DCPP) but contains an additional chlorine atom on the ring without the hydroxyl group. Based on a comparison of physic-chemical and ecotoxicological data on DCPP and Triclosan, it can be concluded that both substances have similar physico-chemical properties. The toxicity of Triclosan is higher than that of DCPP. Therefore, the data on Triclosan can be used to fill data gaps for DCPP. This approach can be regarded as a worst-case approach. For a detailed assessment of this read-across approach see the separate document "Justification of the analogue approach".

1.1	**Reference**	[redacted]2010) [redacted](Triclosan) Effects on Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (Gamasida: Laelapidae), [redacted] Study report Nr. [redacted] Mar-2010
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since submitter is data owner
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD TG 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, October 2008
2.2	**GLP**	Yes (with Certificate)
2.3	**Deviations**	No

3 METHOD

3.1	**Test material**	[redacted](Triclosan)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	100.4%
3.1.4	Composition of Product	See purity
3.1.5	Further relevant properties	White solid powder, stored at room temperature at about 20 °C, away from direct sunlight; stability in water: 0.012 g/L at 20 °C at pH 7 0.0065 g/L at 20 °C at pH 5
3.1.6	Method of analysis	The analytical measurement of the test item was based on HPLC/MS following extraction with acetone/hexane
3.2	**Reference substance**	Yes, Roxion batch Nr. G339A containing 37.5% (i.e. 375 g/L) Dimethoate as active ingredient.

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

3.3 Testing procedure

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

3.3.1 Preparation of the test substance	No aqueous application solution could be prepared due to the low water solubility of the test item, but the test item was highly soluble in the organic solvent acetone (Sponsor's information). Thus, Triclosan was dissolved in acetone and mixed with an adequate amount of quartz sand. For the highest test item application solution, 800 mg of Triclosan were dissolved in 10 mL Acetone, corresponding to 320 mg test item/kg dry soil. For each test concentration, 4 mL of the corresponding application solution was applied to 20 g sand in a mortar. The solvent was completely evaporated at room temperature under a hood in 1 hour. The remainder (sand/test item mixture) was intensively mixed by means of a pestle and then mixed on a roller mixer for 30 minutes.
3.3.2 Application of the test substance	Immediately before the test start, 10 g of each sand/test item mixture (corrected for the amount of test item applied to 10 g of sand) were incorporated into the artificial soil prepared for each test concentration. From the so prepared batches for all test item concentrations, each test vessel was filled with 20 g wet weight of the referring prepared soil.
3.3.3 Reference substance	The reference item treatment was prepared by solving 40 µL Roxion® ad 200 ml purified water, corresponding to 40 mg/kg dry soil. In the reference treatment the same artificial soil as for Triclosan was used.
3.3.4 Test organisms	For details on tested organisms see table A7_5_1_1-1
3.3.5 Test system	For details on test type, laboratory equipment, test substrate, replicates etc. see table A7_5_1_1-2
3.3.6 Test conditions	For details on relevant test conditions see table A7_5_1_1-3
3.3.7 Test duration	The duration of the study was 51 days, divided into 35 days of synchronization, 14 days of exposure and 2 days of extraction.
3.3.8 Test parameter	Mortality, reproduction
3.3.9 Examination	Mortality: At the end of the exposure phase (day 14), each test vessel was set up into a Tullgren funnel assembly. This heat extraction method forced surviving mites to leave the soil because of the increasing temperature which became intolerable at a certain point for the mites. Throughout two days, the temperature within the extraction assembly was increased by 10 °C per day, with an initial temperature of 25 °C and a maximum temperature of 45 °C. The mites from each replicate were trapped in a mixture of purified water and 70% ethanol. The numbers of living adults at the end of the test were compared to the control. Reproduction: The number of juvenile mites of each replicate was determined by manually counting the extracted offspring. The total numbers of juveniles at the end of the test were compared to the control.
3.3.10 Monitoring of test substance concentration	Yes, day 0 and day 14
3.3.11 Statistics	The numbers of living adults at the end of the test were compared to the control and were statistically evaluated by means of a Fischer's exact binomial test with Bonferroni correction (one-sided, greater, $\alpha = 0.05$). The total numbers of juveniles at the end of the test were compared to the control and were statistically evaluated by means of a multiple Williams' t-test. The NOEC and LOEC for reproduction were determined according to the results of the statistics. The EC50 for reproduction was calculated by Weibull Analysis using maximum likelihood regression.

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4 RESULTS

4.1 Analytical monitoring of test concentrations

Performed

4.1.1 Summary of analytical results

Nominal test item treatment (mg/kg dry soil)	Measured test item treatment* (mg/kg dry soil)
Control	Control
0.32	0.29
3.2	1.96
10	6.7
32	23
100	83
320	254
Reference	Reference

4.2 Toxicity findings

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.2.1 Mortality

4.2.1.1 Number of living soil mites and % mortality after 2 weeks

Nominal test item concentration (mg/kg dry soil)	measured test item concentration* (mg/kg dry soil)	Vessel No.	No. of female mites exposed	No. of living females after 2 weeks	Sum of dead female mites	% mortality (%)	±	SD	% mortality corr.*	Stat.*
Control	Control	1 2 3 4 5 6 7 8	10 10 10 10 10 10 10 10	10 10 9 10 7 9 10 10	5	6.3	±	1.1	—	—
0.32	0.29	1 2 3 4	10 10 10 10	9 10 9 10	2.0	5.0	±	0.6	-1.3	n.s.
3.2	1.96	1 2 3 4	10 10 10 10	10 7 7 9	7.0	18	±	1.5	12	n.s.
10	6.7	1 2 3 4	10 10 10 10	9 8 9 8	6.0	15	±	0.6	9	n.s.
32	23	1 2 3 4	10 10 10 10	7 9 6 8	10	25	±	1.3	20	s.
100	83	1 2 3 4	10 10 10 10	7 8 6 7	12	30	±	0.8	25	s.
320	254	1 2 3 4	10 10 10 10	4 0 3 2	31	78	±	1.7	76	s.
Reference	Reference	1 2 3 4	10 10 10 10	0 0 0 1	39	98	±	0.5	97	n.a.

* Statistical comparison of the numbers of alive animals (per treatment): results of a Fischer's exact binomial test with Bonferroni correction, one-sided greater, α=0.05.
n. s.: mean value not statistically significantly greater than the control.
s.: mean value statistically significantly greater than the control.
n.a.: not applicable.
a: Mortality corrected by means of the formula of Abbott [6] with improvements by Schneider-Orelli [7]. A negative corrected mortality value indicates a lower mortality in the test item treatment than in the control.
#: arithmetic mean of the measured test item concentration taken from Sponsor's analytical report (study number for correspondence: 09.259).

4.2.1.2 Treatment-related mortality

In the control group, the mean mortality rate of the adult test organisms after 14 days was 6.3%. The corrected mortality rate increased from -1.3% at the lowest mean measured test concentration of 0.29 mg Triclosan/ kg dry soil (nominal test concentration: 0.32 mg Triclosan/ kg dry soil) to 76% at the highest mean measured test concentration of 254 mg /kg dry soil (nominal test concentration: 320 mg /kg dry soil).

4.3 Reproduction

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)
Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

4.3.1 Reproduction of *Hypoaspis aculeifer*

Nominal test item conc. (mg/kg dry soil)	measured test item concentration# (mg/kg dry soil)	Test Vessel No.	No. of living females	Number of juvenile soil mites per test vessel						Number of juvenile soil mites per surviving female					
				n	Mean	+ SD	CV	% of control	Stat.*	n	Mean	+ SD	CV	% of control	Stat.*
Control	Control	1 2 3 4 5 6 7 8	10 10 9 10 7 9 10 10	109 98 102 121 87 113 107 96	104	11	10	---	---	10.9 9.8 11.3 12.1 12.4 12.6 10.7 9.6	11	1.1	10	---	---
0.32	0.29	1 2 3 4	9 10 9 10	114 110 88 123	109	15	14	104	n.s.	12.7 11.0 9.8 12.3	11	1.3	12	102	n.s.
3.2	1.96	1 2 3 4	10 7 7 9	118 71 58 103	88	28	32	84	n.s.	11.8 10.1 8.3 11.4	10	1.6	15	93	n.s.
10	6.7	1 2 3 4	9 8 9 8	86 67 94 78	81	12	14	78	s.	9.6 8.4 10.4 9.8	10	0.9	9	85	n.s.
32	23	1 2 3 4	7 9 6 8	47 93 39 69	62	24	39	60	s.	6.7 10.3 6.5 8.6	8	1.8	22	72	s.
100	83	1 2 3 4	7 8 6 7	63 44 63 59	57	9.0	16	55	s.	9.0 5.5 10.5 8.4	8	2.1	25	75	s.
320	254	1 2 3 4	4 0 3 2	13 0 19 8	10	8.0	80	10	s.	3.3 0.0 6.3 4.0	3	2.6	77	30	s.
Reference	Reference	1 2 3 4	0 0 0 1	0 2 0 2	1.0	1.2	115	1.6	---	0.0 n.a. 0.0 2.0	1	1.4	141	12.4	---

Note: Calculations were performed with a commercial computer program (EXCEL). The results given in the table are rounded values.
* Statistical comparison of the mean reproduction rate (per treatment):
results of a Williams-t test, one-sided smaller, α=0.05.
n. s.: mean value not significantly different to the control.
s.: mean value significantly different to the control.
n.a.: not applicable because no adults survived after 14 days of exposure.
#: arithmetic mean of the measured test item concentration taken from Sponsor's analytical report (study number for correspondence: 09.259).

4.3.2 Reproduction rate

In the control, an average of 104 juvenile mites per test vessel was found. The coefficient of variance of the reproduction rate in the control was 10%.
In the mean measured test item treatments, the mean reproduction rates per test vessel decreased in a concentration - effect relationship from 104% at 0.29 mg Triclosan/kg dry soil to 10% at 254 mg/kg dry soil of the control value, corresponding to 104% at 0.32 mg/ kg dry soil to 10% at 320 mg/kg dry soil of the control value for the nominal test concentrations. The mean reproduction rates per test vessel were statistically significantly reduced from the mean measured test item concentration of 6.7 mg Triclosan/kg dry soil (nominal test concentration: 10 mg/kg dry soil) up to the highest mean measured test item concentration of 254 mg/kg dry soil (nominal test concentration: 320 mg/kg dry soil).

4.4 NOEC/LOEC, EC50

4.4.1 NOEC and LOEC

Triclosan had a statistically significant toxic effect on the reproduction rate of the predatory soil mites at the mean measured test item concentration of 6.7 mg/kg dry soil (nominal test concentration 10 mg/kg dry soil) up to and including the highest mean measured test concentration of 254 mg/kg dry soil (nominal test concentration 320 mg/kg dry soil) after the test period of 14 days. Therefore, the NOEC according to the mean measured test concentrations (highest test item concentration at which no significant inhibition of the reproduction rate on the test organisms is observed) was determined to be 1.96 mg/kg dry

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)
Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

		soil (3.2 mg/kg dry soil according to the nominal test concentrations). The LOEC according to the mean measured test concentrations (lowest test concentration at which a significant toxic effect on the test organisms is observed) was therefore determined to be 6.7 mg per kg dry soil (10 mg/kg dry soil according to the nominal test concentrations).
4.4.2	EC_{50}	The EC_{50} according to the mean measured test concentrations for reproduction per test vessel was calculated to be 52.3 mg Triclosan/kg dry soil (95% confidence interval: 17.5–134.6 mg Triclosan/kg dry soil). Referring to the nominal test concentrations, the EC_{50} for reproduction per test vessel was calculated to be 68.2 mg/kg dry soil (95% confidence interval: 28.1–151.1 mg/kg dry soil).
4.5	**Test with reference substance**	Performed
4.5.1	Concentrations	The reference item treatment was prepared by solving 40 µL Roxion® ad 200 ml purified water, corresponding to 40 mg/kg dry soil.
4.5.2	Results	At a single concentration of 40 mg/kg dry soil, the reference ite Roxion® resulted in a corrected mortality rate of 97%, The reproduction per test vessel in the reference item treatment was determined to be 1.6% of the control, which is sufficient according to the guideline (at least a reduction of reproduction of 50% compared to the control).

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	The toxic effects of Triclosan on survival and reproduction of the soil predatory mite *Hypoaspis aculeifer* were assessed during a test period of 14 days based on the OECD Guidelines for Testing Chemicals, Test No. 226 (2008); the study conduct was GLP conform. Test substance: Triclosan, Batch Nr. [redacted] purity 100.4% Test system: soil predatory mite *Hypoaspis aculeifer* The test item was homogeneously mixed into artificial soil at the following nominal test concentrations: 0.32, 3.2, 10, 32, 100 and 320 mg/kg dry soil. The analytical monitoring of the test concentration revealed mean measured test item concentrations of 0.29, 1.96, 6.7, 23, 83 and 254 mg/kg dry soil, respectively. Additionally, a negative control and a reference item treatment with 40 mg/kg dry soil of Roxion® were tested in parallel. Female mites were exposed in the treated soils for a period of 14 days. After this period, the numbers of living inserted female adults and of juvenile mites (offspring) were determined.
5.2	**Results and discussion**	In the control group, the mean mortality rate of the adult test organisms after 14 days was 6.3%. The corrected mortality rate increased from -1.3% at the lowest mean measured test concentration of 0.29 mg Triclosan/ kg dry soil (nominal test concentration: 0.32 mg Triclosan/ kg dry soil) to 76% at the highest mean measured test concentration of 254 mg /kg dry soil (nominal test concentration: 320 mg/kg dry soil). In the control, an average of 104 juvenile mites per test vessel was found. The coefficient of variance of the reproduction rate in the control was 10%. In the mean measured test item treatments, the mean reproduction rates per test vessel decreased in a concentration - effect relationship from 104% at 0.29 mg Triclosan/kg dry soil to 10% at 254 mg/kg dry soil of

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)
Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

		the control value, corresponding to 104% at 0.32 mg/ kg dry soil to 10% at 320 mg/kg dry soil of the control value for the nominal test concentrations. The mean reproduction rates per test vessel were statistically significantly reduced from the mean measured test item concentration of 6.7 mg Triclosan/kg dry soil (nominal test concentration: 10 mg/kg dry soil) up to the highest mean measured test item concentration of 254 mg/kg dry soil (nominal test concentration: 320 mg/kg dry soil).
5.2.1	NOEC	1.96 mg/kg dry soil (nominal concentration 3.2 mg/kg dry soil)
5.2.2	LOEC	6.7 mg/kg dry soil (nominal concentration 10 mg/kg dry soil)
5.2.3	EC_{50}	52.3 mg/kg dry soil (confidence interval: 17.5-134.6 mg/kg dry soil), the EC_{50} value for the reproduction rate based on nominal test concentrations was calculated to be 68.2 mg/kg dry soil (confidence interval: 28.1-151.1 mg/kg dry soil).
5.3	**Conclusion**	Triclosan had a toxic effect on the survival and reproduction rates of the soil predatory mites *Hypoaspis aculeifer* from the mean measured test concentration of 6.7 mg/kg dry soil on (nominal test concentration: 10 mg/kg dry soil) after the test period of 14 days; thus, the NOEC was set at 1.96 mg Triclosan/kg dry soil whereas the LOEC was 6.7 mg/kgdry soil. The EC_{50} value for the reproduction rate was 52.3 mg/kg dry soil.
5.3.1	Other Conclusions	In the control group, the mean mortality rate of the adult test organisms after 14 days was 6.3%. Thus, the validity criterion of the test guideline requiring that the mean mortality of the adults in the control should not exceed 20% at the end of the test was fulfilled. In the control, an average of 104 juvenile mites per test vessel was found. The coefficient of variance of the reproduction rate in the control was 10%. Thus, the validity criteria of the test guideline (reproduction per test vessel: at least 50 juveniles with coefficient of variance ≤30%) were well fulfilled. Furthermore, the reproduction rate per test vessel in the reference item treatment was determined to be 1.6% of the control, which is sufficient according to the guideline (at least a reduction of reproduction of 50% compared to the control); the result for the reference item indicates satisfactory test conditions.
5.3.2	Reliability	The study was guideline-conform, the validity criteria were fulfilled, and the test conduct followed GLP (certificate included in the report), thus, a reliability of 1 was given.
5.3.3	Deficiencies	None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	July 2013
Materials and Methods	The applicant's version is acceptable for NOEC determination. However, for determination of NOEC, the concentrations should be spaced by a factor not exceeding 2.0. For a combined approach for determination of both the NOEC and the ECx eight treatment concentrations in a geometric series should be used. The concentrations should be spaced by a factor not exceeding 1.8 in this case.
Results and discussion	Adopt applicant's version for the NOEC value.
Conclusion	NOEC is 1.96 mg/kg (dw)

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

0 READ-ACROSS JUSTIFICATION

Reliability	2
Acceptability	Acceptable for environmental risk assessment
Remarks	While the read across is supported, it has to be stated that DCPP is of similar and not lower toxicity. The approach is therefore a realistic but not a worst case approach.

Table A7_5_1_1-1: Test organisms

Criteria	Details
Species/strain	*Hypoaspis aculeifer* (Gamasida: Laelapidae)
Source of the initial stock	Commercial supplier; the soil predatory mites are successfully bred at [redacted]
Culturing techniques	As prey organisms, mould mites (*Tyrophagus putrescentiae*) were obtained by the same commercial supplier and also successfully bred at [redacted] At the beginning of the test and on day 2, 5, 7, 9 and 12 after application, a sufficient amount of prey organisms (*Tyrophagus putrescentiae*) was added onto the soil surface of each test vessel in order to provide the test organisms with food *ad libitum*.
Age	The soil predatory mites were 28–35 days old
Synchronized culture	Yes, the test organisms, 28–35 days old soil predatory mites, were obtained from a synchronized culture maintained at [redacted] 20 adult female mites were transferred in freshly prepared breeding containers for egg laying over a three-day period. Then, adults were removed. Hatching of the juveniles from the eggs started three to four days after oviposition. After a minimum of 28 days after the first juveniles had hatched, they were used for the test.

Table A7_5_1_1-2: Test system

Criteria	Details
Artificial soil test substrate	The artificial soil was prepared according to the guidelines with the following constituents (percentage distribution on dry weight basis): - Sphagnum peat: 5% (shredded, sieved through 2 mm sieve) - Kaolinite clay: 20% (containing > 30% Al_2O_3) - Sand (Sihelco 36): 75% (Size analysis: 1% (0.25 mm), 6% (0.18 mm), 80% (0.125 mm), 12% (0.09 mm), 1% (<0.09 mm) - Calcium carbonate ($CaCO_3$): 0.3% (for the adjustment to pH 6.0 ± 0.5)
Test mixture	The artificial soil (without calcium carbonate) was prepared in a batch of 20 kg by intensely mixing 1 kg sphagnum peat, 4 kg kaolinite clay, and 15 kg sand (dry weight basis) in a cement mixer. For each test concentration, 513 g of the prepared air dry substrate (503 g substrate + 10 g sand/test item mixture, corresponding to 500 g dry weight) was thoroughly mixed in a laboratory mixer with 1.6 g calcium carbonate and 107 mL purified water. By addition of this amount of purified water, the moisture content of the test substrate was adjusted to approximately 24%, corresponding to approx. 59% of the total water holding capacity (WHC) of the artificial soil. The WHC of the artificial soil amounted to 40.8 g water per 100 g dry soil. The thoroughly mixed test substrate of each test concentration was loosely filled into the test vessels to preserve a crumbly structure enabling the soil mites to penetrate into substrate cavities. The solvent control substrate was prepared in the same way including adjustment of the moisture content but without addition of the test item. Contrary to the solvent control and the test item, the reference item treatment was prepared by solving Roxion® in purified water and adding enough of this solution to the artificial soil in order to reach the assumed soil moisture content.
Size, volume and material of test container	Glass flasks with a volume of 50 mL were used as test vessels; the vessels were labeled with the study number and all necessary additional information to ensure unique identification
Amount of substrate/ container	Each test vessel was provided with approximately 20 g wet mass of the test substrate.
Nominal levels of test concentrations	0.32, 3.2, 10, 32, 100 and 320 mg/kg dry soil
Number of replicates/concentration	For each test item concentration and for the reference item treatment, four replicates (test vessels) were tested. For the solvent control, eight replicates were tested.
Number of organisms/test concentration	A total of 40 mites per test concentration for test item concentration and for the reference item were used. A total of 80 mites were used for the solvent control.
Number of organisms /container	For each test item concentration, for the reference item treatment and for the solvent control, ten adult female mites per vessel were used.
Light source	Not specified
Test performed in closed vessels due to significant volatility of test substrate	The vessels were tightly closed. To allow aeration all test vessels were briefly opened at least twice a week.

Table A7_5_1_1-3: Test conditions

<table>
<tr><th>Criteria</th><th>Details</th></tr>
<tr><td>Test temperature</td><td>The test temperature during the exposure phase (20 °C ± 2 °C) and during the extraction phase (rising over two days from 25 °C to 45 °C) was continuously recorded.
During the entire exposure period, the mean temperature was 22 °C, with a minimum of 21.4 °C and a maximum of 22.4 °C. The temperature was temporarily between 22 and 23 °C for a few hours due to technical difficulties. These slight deviations are considered to have no impact on the reliability of the results or the integrity of the study. During the extraction phase, temperature was risen over a period of two days from initially 23.7 °C to a maximum of 45.1 °C.</td></tr>
<tr><td>Moisture content</td><td>Two additional test vessels (without test organisms) were prepared for the solvent control, each test item concentration and the reference item treatment for determination of pH and soil moisture at test start and at the end of the test. The water holding capacity (WHC) was determined according to ISO guideline 11268-2). For the test, the moisture of the test substrates was adjusted to 24%. At the start of the test, the soil moisture was checked in additional set up soil samples (without mites) of the control, all test item concentrations and the reference item treatment. The measured soil moisture in all samples was 24%, corresponding to 59% of the total water holding capacity of the artificial soil. All test vessels with mites were weighed. To reduce evaporation, the test vessels were tightly closed during the test. After two weeks, the water content was checked by reweighing the additional test containers. The water loss did not exceed 2%. At the end of the test, the soil moisture was checked again for the control, all treatment groups and the reference item treatment.
Water Content and pH of the Artificial Soil:
<table>
<tr><th rowspan="2">Nominal test item treatment (mg/kg dry soil)</th><th rowspan="2">Measured test item treatment[a] (mg/kg dry soil)</th><th colspan="2">Start of the test</th><th colspan="2">After 2 weeks</th></tr>
<tr><th>pH</th><th>Water content (%)</th><th>pH</th><th>Water content (%)</th></tr>
<tr><td>Control</td><td>Control</td><td>6.13</td><td>23.2</td><td>6.31</td><td>23.7</td></tr>
<tr><td>0.32</td><td>0.29</td><td>6.05</td><td>24.9</td><td>6.25</td><td>25.5</td></tr>
<tr><td>3.2</td><td>1.96</td><td>6.07</td><td>24.1</td><td>6.26</td><td>24.6</td></tr>
<tr><td>10</td><td>6.7</td><td>6.08</td><td>24.4</td><td>6.19</td><td>24.1</td></tr>
<tr><td>32</td><td>23</td><td>6.25</td><td>24.5</td><td>6.22</td><td>24.4</td></tr>
<tr><td>100</td><td>83</td><td>6.15</td><td>24.1</td><td>6.10</td><td>23.9</td></tr>
<tr><td>320</td><td>254</td><td>6.09</td><td>24.2</td><td>6.09</td><td>24.5</td></tr>
<tr><td>Reference</td><td>Reference</td><td>6.14</td><td>24.9</td><td>6.06</td><td>24.6</td></tr>
</table>
The soil moisture content was 23– 25% at test start and 24– 26% at the termination of the test after 2 weeks in the control, all test item treatments and the reference item treatment.</td></tr>
<tr><td>pH</td><td>See above.
At the start of the test, the pH was 6.1 - 6.3 in all treatments. At the end of the exposure phase after 14 days, the pH was 6.1 – 6.3 in all treatments.</td></tr>
<tr><td>Adjustment of pH</td><td>The pH was adjusted by addition of 0.3% calcium carbonate.</td></tr>
<tr><td>Light intensity / photoperiod</td><td>The test vessels were kept under a 16-hour light to 8-hour dark photoperiod with a 30 minutes transition period, and a light intensity during the light period of 688–763 Lux at the substrate surface.</td></tr>
<tr><td>Relevant degradation products</td><td>Not applicable</td></tr>
</table>

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

Official use only

1 REFERENCE

1.1	**Reference**	[redacted] (2014); Effects of Benzene, 4-chloro-1—(chlorophenoxy)-2-methoxy- on the reproduction of the predatory mite, *Hypoaspis aculeifer*; [redacted] project No: [redacted] S; BASF project No.: [redacted] 17-Jun-2014
1.2	**Data protection**	Yes
1.2.1	Data owner	BASF SE
1.2.2	Companies with letter of access	Not applicable since submitter is data owner
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

2 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	Yes, OECD TG 226: Predatory mite (*Hypoaspis (Geolaelaps) aculeifer*) reproduction test in soil, October 2008
2.2	**GLP**	Yes (with Certificate)
2.3	**Deviations**	None

3 METHOD

3.1	**Test material**	Benzene, 4-chloro-1—(chlorophenoxy)-2-methoxy- (methyl-diclosan, Me-O-DCPP)
3.1.1	Lot/Batch number	[redacted]
3.1.2	Specification	As given in section 2
3.1.3	Purity	> 99.5%
3.1.4	Composition of Product	Technical substance
3.1.5	Further relevant properties	- White solid powder, stored dry at room temperature at about 20 °C - Molecular weight: 269.127 g/mol - Water solubility: 0.322 mg/L - stable under the given conditions
3.1.6	Method of analysis	Analytical measurement was not performed. Not required by the guideline.
3.2	**Reference substance**	Dimethoate (analysed purity: 99.8 %, tolerance ± 1.0 %). (see ch. 4.5)
3.3	**Testing procedure**	

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

3.3.1 Preparation of the test substance	No aqueous application solution could be prepared due to the low water solubility of the test item, but the test item was highly soluble in the organic solvent acetone (Sponsor's information). Thus, Methyl-Diclosan was exactly weighed and dissolved in acetone as a stock solution. This stock solution was stepwise diluted with acetone to prepare 10 further test solutions (serial dilution; spacing factor 1.8). Afterwards, the test solutions (4 mL each) were thoroughly mixed with finely ground quartz sand (8 g each) to prepare 11 test item mixtures of different test item concentrations. After preparation of the test item mixtures, the acetone was removed by evaporation in a fume hood for at least one hour. The test item mixtures were then mixed with the prepared artificial soil by intensive stirring. Additionally, a solvent control was prepared with the same amount of the solvent (acetone) as used for the concentration levels.	
3.3.2 Application of the test substance	Immediately before the test start, the amount of the artificial soil per concentration was weighed; the test item mixture was added to the soil substrate, the test substrate was thoroughly mixed and 20 g soil dry weight were introduced into each test vessel avoiding compression.	
3.3.3 Reference substance	Details are reported in a separate study for the reference item (BioChem project No. R 14 10 48 001 S, dated June 10, 2014)	
3.3.4 Test organisms	For details on tested organisms see table A7_5_1_1-1	x x
3.3.5 Test system	For details on test type, laboratory equipment, test substrate, replicates etc. see table A7_5_1_1-2	
3.3.6 Test conditions	For details on relevant test conditions see table A7_5_1_1-3	x x x
3.3.7 Test duration	The total time of the study was 51 days, divided into 35 days of synchronization, 14 days of exposure and 2 days of extraction.	
3.3.8 Test parameter	Mortality, reproduction	
3.3.9 Examination	14 days after application of the test item and introduction of the test organisms, surviving mites and juveniles of *Hypoaspis aculeifer* were extracted from each test replicate using a MacFadyen high-gradient extractor (heat/light extraction method). This was achieved by adding the soil substrate from each test vessel into a canister placed inverted onto the extraction system. Soil substrate was retained within the canister using a plastic net (1 mm mesh size). Beneath the canister was a funnel attached to a collecting flask with 25 mL of a fixing liquid. A temperature gradient was created between the upper part and the lower part of the system. The temperature gradient was obtained by circulating heated air in the canister area and cooled air on the collecting area. The duration of extraction was 48 h at the following heating regime: 25 °C for 12 h, 35 °C for 12 h, 45 °C for 24 h. During this time, adult and juvenile mites moved down through the soil substrate away from the heat source, until eventually falling from the substrate into the funnel / fixing liquid. Following extraction, all juveniles and adults present in the fixing liquid were counted. Any adult mites not found after extraction were recorded as dead. From these data the mortality of the adult females and the reproductive output were calculated.	

3.3.10 Monitoring of test substance concentration	Not performed. Not required by the guideline.
3.3.11 Statistics	- Mortality: Mortality (number of dead adults) in % for each treatment group was calculated. Missing mites were counted as dead. - Reproduction: The reproductive output for each treatment group was calculated in % compared to solvent control. The statistical analysis was performed with the software ToxRat Professional 2.10.05 (Ratte 2010). Fisher's Exact Binomial Test with Bonferroni correction and Williams-t-test were used to compare the solvent control with the independent test item groups. Probit analysis was used for EC10, EC20 and EC50 estimation

4 RESULTS

4.1 Analytical monitoring of test concentrations	Not applicable since analytical measurement is not required by the test guideline.
4.1.1 Summary of analytical results	Not applicable since analytical measurement is not required by the test guideline.
4.2 Toxicity findings	

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

4.2.1 Mortality

4.2.1.1 Number of living soil mites and % mortality after 2 weeks

Effects of the test item on mortality of adult mites

Treatment group [mg test item/kg soil dry weight]	Number of surviving adult mites per replicate (14 days after test start) 1	2	3	4	5	6	7	8	Mean	Standard deviation (± SD)	Mortality [%]
Solvent control	10	10	10	8	9	10	10	10	9.6	0.7	3.8
2.8	10	10	10	10	-	-	-	-	10.0	0.0	0.0
5.0	10	10	9	10	-	-	-	-	9.8	0.5	2.5
9.1	10	10	10	10	-	-	-	-	10.0	0.0	0.0
16	10	8	10	10	-	-	-	-	9.5	1.0	5.0
29	9	8	10	10	-	-	-	-	9.3	1.0	7.5
53	10	10	9	10	-	-	-	-	9.8	0.5	2.5
95	10	10	10	10	-	-	-	-	10.0	0.0	0.0
171	10	10	9	9	-	-	-	-	9.5	0.6	5.0
309	10	10	10	8	-	-	-	-	9.5	1.0	5.0
556	10	7	10	10	-	-	-	-	9.3	1.5	7.5
1000	7	10	10	10	-	-	-	-	9.3	1.5	7.5

No statistically significant differences compared to the solvent control were calculated (Fisher's Exact Binomial Test with Bonferroni Correction for mortality, α = 0.05, one-sided greater)

4.2.1.2 Treatment-related mortality

Mortality rates of 0.0 - 7.5 % were recorded in the test item treatment groups. In the solvent control the mortality rate was 3.8 %. The observed mortality rates for adults mortality in the test item treatment groups compared to the solvent control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater).

Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.

The results are summarised in the table above (ch. 4.2.1.1)

4.3 Reproduction

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

4.3.1 Reproduction of *Hypoaspis aculeifer*

Effects of the test item on number of juvenile mites

Treatment group [mg test item/kg soil dry weight]	Number of juvenile mites per replicate (14 days after test start) 1	2	3	4	5	6	7	8	Mean	Standard deviation (± SD)	Coefficient of variation [%]	Reproduction [% of Solvent control]
Solvent control	264	239	184	169	229	186	209	228	214.8	26.7	12.4	100
2.8	234	229	208	204	-	-	-	-	218.8	15.0	6.8	102
5.0	231	188	189	160	-	-	-	-	197.0	23.0	11.7	92
9.1	171	155	180	170	-	-	-	-	171.3*	13.0	8.1	80
16	171	163	166	145	-	-	-	-	161.3*	11.3	7.0	75
29	100	167	145	159	-	-	-	-	162.0*	14.7	9.0	76
53	161	113	134	113	-	-	-	-	127.8*	18.4	14.4	59
95	135	122	70	94	-	-	-	-	105.3*	29.1	27.6	49
171	72	86	89	65	-	-	-	-	78.0*	11.4	14.6	36
309	78	93	57	35	-	-	-	-	65.8*	25.3	38.4	31
556	55	50	58	46	-	-	-	-	52.3*	5.3	10.2	24
1000	29	45	19	41	-	-	-	-	33.5*	11.8	35.3	16

Calculations were done using non-rounded values
* statistically significant differences compared to the solvent control were calculated (Williams-t-test for reproduction; α = 0.05, one-sided smaller)

4.3.2 Reproduction rate

The respective reproduction rates in the 2.8, 5.0, 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight were 218.8, 197.0, 171.3, 161.3, 162.8, 127.8, 105.3, 78.0, 65.8, 52.3 and 33.5 juveniles per replicate, respectively. The mean reproduction in the solvent control reached 214.8 juveniles.

The test item showed no statistically significantly adverse effects on reproduction up to and including 5.0 mg/kg soil dry weight.

However, the test item caused statistically significant effects on reproduction at 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg/kg soil dry weight (Williams-t-test, $\alpha = 0.05$, one-sided smaller).

The results are summarised in the table above (ch. 4.3.1).

4.4 NOEC/LOEC, EC50

4.4.1 NOEC and LOEC

The NOEC for mortality was determined to be 1000 mg test item/kg soil dry weight. The NOEC for reproduction was determined to be 5.0 mg test item/kg soil dry weight.

The EC10 value for reproduction was 5.2 mg test item per kg soil dry weight. x

4.4.2 EC_{50}

The EC50 for reproduction was determined to be 94.8 mg test item/kg soil dry weight. The LC50 for mortality could not be calculated, but it can be concluded that the LC50 is higher than 1000 mg test item/kg soil dry weight, the highest concentration tested. x

4.5 Test with reference substance

Performed

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

4.5.1	Concentrations	The reference item was tested at concentrations of 1.00, 1.60, 2.56, 4.10, 6.55 and 10.5 mg test item/kg soil dry weight.
4.5.2	Results	In a separate study (BioChem project No. R 14 10 48 001 S, dated June 10, 2014), the EC50 (reproduction) of the reference item Dimethoate was calculated to be 6.2 mg test item/kg soil d.w. The results of the reference test demonstrate the sensitivity of the test system.

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The effects of Methyl-Diclosan on mortality and reproduction of the predatory mite *Hypoaspis aculeifer* (CANESTRINI) were investigated in a chronic laboratory experiment over a time period of 14 days according to OECD 226 (2008). The test item was dissolved in acetone and applied onto quartz sand. This treated quartz sand was mixed into artificial soil at concentrations of 2.8, 5.0, 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight. For the control treatment, the soil was prepared with acetone (solvent control). 8 replicates and 4 replicates were prepared for the control and test item treatment groups, respectively, each containing 10 adult predatory mites (females). Assessment of mortality and reproduction was carried out after the 14-day exposure of the predatory mites.

Test substance:

Benzene, 4-chloro-1--(4-chlorophenoxy)-2-methoxy- (Methyl-Diclosan); Batch No.: [redacted] purity: > 99.5 %

Test system: soil predatory mite *Hypoaspis aculeifer*

The different concentrations of the test item were homogeneously mixed into the artificial soil which was then used to fill glass vessels after which the predatory mites were introduced on top of the soil; 12 treatment groups (11 test item concentrations, solvent control); 8 replicates/solvent control group and 4 replicates/test item treatment group each with 10 predatory mites. Feeding of mites with *Tyrophagus putrescentiae* (SCHRANK) at the beginning and ad libitum during the test.

Assessment of adult mortality and reproduction effects was carried out after 14 days.

5.2 Results and discussion

Mortality rates of 0.0 - 7.5 % were recorded in the test item treatment groups. In the solvent control the mortality rate was 3.8 %. The observed mortality rates for adults mortality in the test item treatment groups compared to the solvent control were not statistically significant (Fisher's Exact Binomial Test with Bonferroni Correction, $\alpha = 0.05$, one-sided greater). Differences between the behaviour and the morphology of the mites in the solvent control and the test item treatment groups could not be observed.

Reproduction rates in the 2.8, 5.0, 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight were 218.8, 197.0, 171.3, 161.3, 162.8, 127.8, 105.3, 78.0, 65.8, 52.3 and 33.5 juveniles per replicate, respectively. The mean reproduction in the solvent control reached

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

		214.8 juveniles per replicate. The test item showed no statistically significantly adverse effects on reproduction up to and including 5.0 mg/kg soil dry weight. However, the test item caused statistically significant effects on reproduction at 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg/kg soil dry weight (Williams-t-test, α = 0.05, one-sided smaller)	
5.2.1	NOEC	The NOEC for mortality was determined to be 1000 mg test item/kg soil d. w. The NOEC for reproduction was determined to be 5.0 mg test item/kg soil d. w.	
5.2.2	LOEC	The LOEC value for reproduction was 9.1 mg test item/kg soil d. w.	
5.2.3	EC_{50}	The EC50 for reproduction was determined to be 94.8 mg test item/kg soil dry weight. The LC50 for mortality could not be calculated, but it can be concluded that the LC50 is higher than 1000 mg test item/kg soil dry weight.	x
5.3	**Conclusion**	In a 14-day *Hypoaspis aculeifer*-reproduction study with Benzene, 4-chloro-1-(4-chlorophenoxy)-2-methoxy- (Methyl-Diclosan), the test item showed no statistically significantly adverse effects on mortality. In case of reproduction the test item showed no statistically significantly adverse effects up to and including 5.0 mg/kg soil dry weight. However, the test item caused statistically significant effects on reproduction at concentrations ≥ 9.1 soil dry weight (Williams-t-test, α = 0.05, one-sided smaller).	
5.3.1	Other Conclusions	In the control group (solvent control), the mean mortality rate of the adult test organisms after 14 days was 3.8%. Thus, the validity criterion of the test guideline requiring that the mean mortality of the adults in the control should not exceed 20% at the end of the test was fulfilled. In the control, an average of 214.8 juvenile mites per replicate was found. The coefficient of variance of the reproduction rate in the control was 12.4%. Thus, the validity criteria of the test guideline (reproduction per test vessel: at least 50 juveniles with coefficient of variance ≤30%) were well fulfilled. Furthermore, the results for the reference item (separate study (BioChem project No. R 14 10 48 001 S, dated June 10, 2014) indicates a sufficient sensitivity.	
5.3.2	Reliability	The study was guideline-conform, the validity criteria were fulfilled, and the test conduct followed GLP (certificate included in the report), thus, a reliability of 1 was given.	
5.3.3	Deficiencies	None	

Section A7.5.2.1 Reproduction of the Soil Predatory Mite *Hypoaspis aculeifer* (OECD TG 226)

Annex Point IIIA XIII 3.2

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2014
Materials and Methods	3.3.4 *Correction*: table A7_5_1_1-1: Age: The soil predatory mites were ~~28- 35~~ 33-35 days old from a synchronised culture with an age difference of 3 days. 3.3.4 *Correction*: table A7_5_1_1-1: Synchronized culture: Yes, the test organisms, ~~28- 35~~ 33-35 days old soil predatory mites, were obtained from a synchronized culture maintained at the test facilities. 3.3.6 *Correction*: table A7_5_1_1-3: Test temperature: The test temperature during the exposure phase was measured to be constantly at 20.7 °C, with a minimum of ~~21.7~~ 20.7 °C and a maximum of 20.8 °C. 3.3.6 *Correction*: table A7_5_1_1-3: Moisture content: The water loss did not exceed ~~1%~~ 10%. *(maximum water loss was 1.3%)* 3.3.6 *Correction*: table A7_5_1_1-3: pH: At the start of the test, the pH was ~~5.1~~ 5.7 – 5.9 in all treatments.
Results and discussion	4.4.1 *Addition*: The EC10 value for reproduction was 5.2 mg (95% CI: 3.4-7.9 mg) test item per kg soil dry weight. 4.4.2 *Addition*: The EC50 for reproduction was determined to be 94.8 mg (95% CI: 78.1-115.1 mg) test item/kg soil dry weight.
Conclusion	5.2.3 *Addition*: The EC50 for reproduction was determined to be 94.8 mg (95% CI: 78.1-115.1 mg) test item/kg soil dry weight.
Reliability	1
Acceptability	Acceptable with the amendments given above.
Remarks	-

Table A7_5_1_1-1: Test organisms

Criteria	Details
Species/strain	*Hypoaspis aculeifer* (Gamasida: Laelapidae)
Source of the initial stock	Originally obtained from "Bayer CropScience AG" Monheim; reared in the test facility under ambient laboratory conditions
Culturing techniques	The test organisms were kept at room temperature (approx. 20 °C) under the light conditions light : dark = 16 h : 8 h (artificial light; 516 lx). As prey organisms served mould mites, *Tyrophagus putrescentiae* (Schrank); the food was supplied twice to three times a week.
Age	The soil predatory mites were 28–35 days old from a synchronised culture with an age difference of 3 days.
Synchronized culture	Yes, the test organisms, 28–35 days old soil predatory mites, were obtained from a synchronized culture maintained at the test facilities. Adult female mites were transferred in freshly prepared breeding containers for egg laying over a three-day period. Then, adults were removed. Subsequently, the adult mites were removed and the rearing vessels with the remaining eggs, which had been laid during the last 3 days, were incubated for further 32 days (at approximately 20 °C). Within this incubation time the laid eggs developed to adult mites of similar age. At day 35 after transfer of the parental females to the rearing vessels for egg-laying, the mites of the synchronised culture were suitable for the test.

Table A7_5_1_1-2: Test system

Criteria	Details
Artificial soil test substrate	The artificial soil was prepared according to the guidelines with the following constituents (percentage distribution on dry weight basis): • Sphagnum peat: 5% ; origin: Torfwerk Moorkultur Ramsloh, 26683 Saterland, Germany, classified according to DIN 11540 (as close to pH 5.5-6.0 as possible, no visible plant remained, finely ground, dried to measured moisture content) • Kaolinite clay: 20% (kaolinite content > 30 %); type: Kaolin W, origin: ERBSLÖH Lohrheim GmbH, 65558 Lohrheim, Germany • Industrial quartz sand: 74.6% type: Millisil W3, origin: Quarzwerke GmbH, 50207 Frechen, Germany (fine sand is dominant with more than 50 % of the particles between 50 and 200 µm) • Calcium carbonate (CaCO3): 0.2% origin: MERCK KGaA, 64271 Darmstadt, Germany • Deionised Water
Test mixture	Methyl-Diclosan was exactly weighed and dissolved in acetone as a stock solution. This stock solution was stepwise diluted with acetone to prepare 10 further test solutions (serial dilution; spacing factor 1.8). Afterwards, the test solutions (4 mL each) were thoroughly mixed with finely ground quartz sand (8 g each) to prepare 11 test item mixtures of different test item concentrations. After preparation of the test item mixtures, the acetone was removed by evaporation in a fume hood for at least one hour. 5 g of the corresponding test item mixture, containing the required amount of the test item, and deionised water (16.56 mL) were added to each prepared amount of artificial soil (218.50 g wet weight + 5 g test item mixture = 200 g soil d.w.), resulting in concentrations of 2.8, 5.0, 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight, with a final nominal water content of about 50 % of maximum WHC. Subsequently, 24.01 g of the treated artificial soil (= 20 g soil dry weight) were placed into each test vessel. For the substrate of the solvent control 10 g with acetone (5 mL) treated quartz sand and deionised water (33.13 mL) were added to the prepared amount of artificial soil (437.00 g wet weight + 10 g with acetone treated quartz sand = 400 g soil d. w.). The acetone was removed by evaporation in a fume hood. The final water content was then checked to be 44.17 - 49.60 % of WHC.
Size, volume and material of test container	100 mL SCHOTT-bottle with screw cap (inside dimensions: 4 cm in diameter, 11 cm high)
Amount of substrate/ container	Each test vessel was provided with approximately 24 g wet mass of the test substrate (= 20 g dry weight)
Nominal levels of test concentrations	2.8, 5.0, 9.1, 16, 29, 53, 95, 171, 309, 556 and 1000 mg test item/kg soil dry weight
Number of replicates/concentration	- Treated group: 4 replicates (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites) - Solvent control group: 8 (+ 2 replicates for determination of water content and pH-value; not loaded with predatory mites)

Number of organisms/test concentration	- Treated group: 40 - Solvent control group: 80
Number of organisms /container	10
Light source	artificial light (Lumilux L58W)
Test performed in closed vessels due to significant volatility of test substrate	All test vessels were tightly closed; the vessels were briefly opened every 2 - 3 days for aeration.

Table A7_5_1_1-3: Test conditions

Criteria	Details
Test temperature	The test temperature during the exposure phase was measured to be constantly at 20.7 °C, with a minimum of 21.7 °C and a maximum of 20.8 °C; The duration of extraction was 48 h at the following heating regime: 25 °C for 12 h, 35 °C for 12 h, 45 °C for 24 h
Moisture content	Two additional test vessels (without test organisms) were prepared for the solvent control and each test item concentration for determination of pH and soil moisture at test start and at the end of the test. The maximum water holding capacity (WHC) was determined to be 40.06 g/100 g soil dry weight. At the start of the test, the soil moisture was checked for all test item concentrations and the control. The measured soil moisture in all samples was between 44.2 – 49.6% of the total WHC. To reduce evaporation, the test vessels were tightly closed during the test. After two weeks, the water content was checked again and determined to be 42.64 – 48.5%. The water loss did not exceed 1%. Water Content and pH of the Artificial Soil: Treatment group [mg test item/kg soil dry weight] / Day 0 [g/100 g soil dry weight] / Day 14 [g/100 g soil dry weight] Solvent control: 19.04 / 18.03 2.8: 19.69 / 18.71 5.0: 19.87 / 18.54 9.1: 19.27 / 18.10 16: 19.22 / 19.01 29: 17.93 / 17.08 53: 19.69 / 19.40 95: 19.39 / 18.82 171: 19.57 / 19.14 309: 19.45 / 18.82 556: 19.81 / 19.43 1000: 17.70 / 17.63
pH	Two additional test vessels (without test organisms) were prepared for the solvent control and each test item concentration for determination of pH and soil moisture at test start and at the end of the test. At the start of the test, the pH was 5.1 – 5.9 in all treatments. At the end of the exposure phase after 14 days, the pH was 5.7 – 6.2 in all treatments. Treatment group [mg test item/kg soil dry weight] / Day 0 / Day 14 Solvent control: 5.92 / 6.20 2.8: 5.78 / 5.93 5.0: 5.78 / 5.81 9.1: 5.76 / 5.80 16: 5.75 / 5.75 29: 5.74 / 5.77 53: 5.78 / 5.71 95: 5.77 / 5.78 171: 5.79 / 5.78 309: 5.75 / 5.79 556: 5.71 / 5.68 1000: 5.73 / 5.72

Adjustment of pH	The pH was adjusted by addition of 0.2% calcium carbonate.
Light intensity /photoperiod	intensity: 518 lx photoperiod: light : dark = 16 h : 8 h

Section A7.5.3.1.2 **Annex Point IIIA XIII.1.2**	**Short-term toxicity on birds**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	A short-term toxicity test with birds is not required for biocidal active substances used as disinfectant (Biocidal Product Types 1, 2 and 4, see TNsG for Data Requirements (April 2000), chapter 2.5). However, a study conducted with the structurally similar active substance triclosan (CAS-No. 3380-34-5) is available. The phenol ring of triclosan and DCPP is identical. Furthermore, since triclosan has a higher degree of chlorination (three chlorine atoms) compared with DCPP (two chlorine atoms), it can be expected that triclosan has a higher potential for bioaccumulation and a higher toxicity to organisms than DCPP. Thus, it is considered justified to bridge the results of the study performed with triclosan to DCPP.	
Undertaking of intended data submission [ ]	–	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE FI	
Date	July 2011	
Evaluation of applicant's justification	Agree with applicant's version.	
Conclusion	Agree with applicant's version.	
Remarks	-	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII.1.2 Bobwhite quail

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted](1993b): Triclosan [redacted]®): 8-Day Acute Dietary LC_{50} Study in Bobwhite Quail. [redacted] Report No. [redacted] date: 1993-04-19 (unpublished).	
1.2	**Data protection**	Yes	
1.2.1	Data owner	BASF SE	
1.2.2	Companies with letter of access	-	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I/IA	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	U.S. EPA FIFRA Guideline Subdivision E, § 71-2 "Acute oral LD_{50}" (1989) in conjunction with U.S. EPA Guideline 540/9-85-008 (1985)	x
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	[redacted] (**Triclosan**)	
3.1.1	Lot/Batch number	Batch No. [redacted]	
3.1.2	Specification	-	
3.1.3	Purity	99.7%	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis in the diet	Triclosan is extracted with methanol from a weighed amount of avian feed for at least 6 hours in a soxhlet apparatus. An aliquot of the extract is filtered using 0.45 micron filters and analysed against standards by reverse phase high performance liquid chromatography (HPLC).	
3.2	**Administration of the test substance**	See table A7_5_3_1_1-1	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		
3.4.1	Test organisms	Bobwhite quail, see table A7_5_3_1_2-2	
3.4.2	Test system	See table A7_5_3_1_2-3	
3.4.3	Diet	See table A7_5_3_1_2-3	

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII.1.2 Bobwhite quail

3.4.4	Test conditions	See table A7_5_3_1_2-4
3.4.5	Duration of the test	Exposure: 5-day observation period when treated diets were administered. Recovery: 3-day post-exposure observation period after treated diets had been removed.
3.4.6	Test parameter	Mortality, symptoms, body weight and feed consumption were monitored. Postmortem examinations were conducted on all mortalities and a percentage of birds sacrificed at study termination.
3.4.7	Examination/ Observation	See table A7_5_3_1_2-3
3.4.8	Statistics	Since there were less than 50 % mortality at the highest concentration tested, an LC_{50} was not calculated and an estimation of the LC_{50} value was made by a visual inspection of the mortality data.
		4 RESULTS
4.1	**Limit Test / Range finding test**	No
4.1.1	Concentration	-
4.1.2	Number/ percentage of animals showing adverse effects	-
4.1.3	Nature of adverse effects	-
4.2	**Results test substance**	
4.2.1	Applied concentrations	The 2500 ppm test diet was prepared by mixing equal amounts (6.5 kg each) of stock diet and 5000 ppm test diet in the Hobart H-600-DT mixer for 30 minutes. Each successively lower test level diet was prepared in a similar fashion by using the next higher test level diet. Nominal dietary concentrations: 312, 625, 1250, 2500, and 5000 mg a.s./kg diet Measured concentrations: Analysis of the 625, 1250, and 2500 ppm dose verification/day 0 stability samples collected immediately after preparation revealed average values of 616.1, 1192, and 2370 ppm, respectively (98.6%, 95.4%, and 94.8%, respectively). Analysis of the 312, 625, 1250, 2500, and 5000 ppm day 5 stability samples collected from the animal room revealed average values of 331.1, 564.3, 1173, 2492, and 5469 ppm, respectively (106%, 90.3%, 93.8%, 99.8%, and 109%, respectively).
4.2.2	Effect data (Mortality)	The following mortalities were recorded during the investigation: one in the 2500 ppm test group and four in the 5000 ppm test group. No mortalities were recorded in 312, 625, and 1250 ppm test groups throughout the investigation. See also table A7_5_3_1_2-5.

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII.1.2 Bobwhite quail

4.2.3	Body weight	The average body weight in the 5000 ppm test group was reduced in comparison to those in the vehicle control groups,on test days 5 and 8. All other average body weights were comparable to those in the vehicle control groups. See table A7_5_3_1_2-6
4.2.4	Feed consumption	Feed consumption values during the last two days of the quarantine period ranged from 4 to 5 grams/bird/day. Reduced feed consumption was noted in the 5000 ppm test group during the first five test days. All other feed consumption values were considered to be normal. See table A7_5_3_1_2-6
4.2.5	Concentration / response curve	Not given in the report
4.2.6	Other effects	No clinical signs of toxicity (other than death) were noted in the test groups throughout the study. Gross pathological examinations of the five birds that died during the test revealed gaseous intestines in two 5000 ppm birds. No other abnormal findings were recorded. Gross pathological examinations of 24 arbitrarily selected survivors at termination revealed no abnormal findings. See also table A7_5_3_1_2-5 and -6.
4.3	**Results of controls**	No mortalities were observed in the solvent control. No clinical signs of toxicity (other than death) were noted in the vehicle control throughout the study.
4.3.1	Number/ percentage of animals showing adverse effects	-
4.3.2	Nature of adverse effects	-
4.4	**Test with reference substance**	Not performed
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Triclosan ([redacted]®) was tested in an 8-day acute dietary LC_{50} study conducted according to the U.S. EPA FIFRA Guideline Subdivision E, § 71-2. Triclosan was fed in the diet at levels of 312, 625, 1250, 2500, and 5000 ppm to five groups of bobwhite quail (*Colinus virginianus*) for a period of five days followed by a three-day recovery period. Each group consisted of ten 13-day-old bobwhite quail of indeterminate sex. Two similarly-sized control groups received stock diet mixed with acetone.
5.2	**Results and discussion**	Five mortalities (one in the 2500 ppm test group and four in the 5000 ppm test group) were recorded throughout the investigation. The average body weight was depressed in the 5000 ppm test group on test

Section A7.5.3.1.2 Short-term toxicity on birds

Annex Point IIIA XIII.1.2 Bobwhite quail

		days 5 and 8. Reduced feed consumption was noted during the first five test days in the 5000 ppm test group.
5.2.1	LC_{50}	LC_{50} > 5000 mg a.s./kg diet
5.2.2	NOEC	NOEC = 1250 mg a.s./kg diet (based on exposure period)
5.3	**Conclusion**	Validity criteria for short-term avian toxicity test according to OECD Guideline 205 are fulfilled (cf. table A7_5_3_1_2-7).
5.3.1	Reliability	1
5.3.2	Deficiencies	No

Evaluation by Competent Authorities

	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	July 2011
Materials and Methods	2.1: Replace “Acute oral LD50” by “Avian dietary LC50 test”
	3.4.1: ad Table A7_5_3_1_2-2:In box for “Breeding population”: Replace “2” by “2 or 5”, as it is understood that some animals were received later.
	3.4.1: ad A7_5_3_1_2-2: in box for “Time and intervals of body weight determination: Replace “3” by “1” (probably typing error).
Results and discussion	Adopt applicant's version.
Conclusion	Adopt applicant's version.
Reliability	1
Acceptability	Acceptable for environmental risk assessment with amendments given above.
Remarks	Housing conditions do not comply with recommendations in OECD 205. Light should not be 24 hours per day, but 12-16 hours. Humidity is too low (26 % compared to the recommended 50-75 %). Temperature (37 °C) is appropriate when the birds are 0-7 days old, but should be lowered to 25-28 °C, when the birds are more than 14 days old. With 278 cm^2/bird the cage space was slightly below the recommended 300 cm^2/bird. If the test substance had been more toxic to birds the stress caused by these test conditions could have been a problem. However, this does not appear to have affected the results of this study.

Table A7_5_3_1_2-1: Method of administration of the test substance

Carrier/Vehicle	Details
Water	No
Organic carrier	Acetone
Concentration of the carrier [% v/v]	At the highest concentration: 65.00 grams Triclosan / 195.0 grams acetone
Other vehicle	-
Function of the carrier / vehicle	Triclosan [redacted], in an amount of 65.00 grams, was added to 195.0 grams acetone and mixed into 7935 grams of a standard laboratory diet to form a "standard premix". The "standard premix" was blended for 15 minutes. An additional 5000 grams of stock diet was then mixed into the "standard premix". This entire mixture was allowed to blend for 15 minutes. This became the 5000 ppm test diet.

Table A7_5_3_1_2-2: Test animals

Criteria	Details
Species/Strain	Bobwhite quail (*Colinus virginianus*)
Source	The birds were purchased from [redacted]
Age, sex and initial body weight (bw)	Age: 13 days at beginning of 5-days exposure period Sex: unknown Body weight: 23 g (average on Day 0).
Breeding population	The birds were only 2-3 days old at the time of their arrival in the test facilities
Amount of food	Throughout acclimation and testing period all test birds were fed a Purina® Game Bird Startena®. Food and water were available ***ad libitum***.
Age at time of first dosing	Age: 13 days-old
Health condition / medication	Thirty-seven birds were found dead during the 11-day quarantine period. No deaths were recorded during the last three days of the quarantine period. All other birds were normal and active during this period. Prior to initiation of the project, all birds were examined and their suitability for testing (based on general physical condition) was determined.

Table A7_5_3_1_2-3: Test system

Criteria	Details
Test location	Indoor, wire pens
Holding pens	45.7 cm x 61.0 cm x 45.7 cm steel wire pens
Number of animals	70 (50 for dose groups, 20 for control groups (10 for control 1: raw feed, 10 for control 2: vehicle/solvent control))
Number of animals per pen [cm²/bird]	10 birds of unknown sex (278 cm²/bird)
Number of animals per dose	Each treatment or control group contained ten birds
Pre-treatment / acclimatisation	One hundred and sixteen birds were placed in quarantine for an 11-day acclimation period to determine their suitability for test and to acclimate them to laboratory conditions. All birds were fed Purina® Game Bird Startena® throughout the acclimation period. Lighting was provided by fluorescent lights which were on 24 hours per day. The animal room temperature (minimum and maximum) during the acclimation averaged 100°F (38°C) and the average relative humidity was 26%.
Diet during test	Test birds received sufficient fresh diet (containing the test material at determined doses) on a daily basis for five consecutive days. After this five-day test period, treated diets were removed and birds were offered untreated feed for a three-day recovery period.
Dosage levels (of test substance)	Birds were exposed for five days to nominal dietary concentrations of 312, 625, 1250, 2500, and 5000 mg a.s./kg diet
Replicate/dosage level	No applicable (ten birds per treatment)
Feed dosing method	Orally by feed
Dosing volume per application	Food was available *ad libitum*
Frequency, duration and method of animal monitoring after dosing	Feed consumption was recorded for each group during the last two days of the quarantine period, at the end of the five-day test period, and during the three-day recovery period. Inspections were made daily for mortalities, abundance of feed and water, and feed spillage. Observations were made daily to ascertain the presence (or absence) of clinical signs indicative of test material effect. Four arbitrarily selected birds from the vehicle control groups and from each of the test groups were subjected to gross pathological examinations at the termination of the project.
Time and intervals of body weight determination	Birds were weighed by groups (not individually) at 0 hour on test day 3 and on test days 5 and 8.

Table A7_5_3_1_2-4: Test conditions (housing)

Criteria	Details
Test temperature	On average: 98°F (37°C) (thermostatically-controlled, heated environment)
Shielding of the animals	No data
Ventilation	No data
Relative humidity	On average: 26 %
Photoperiod and lighting	Lighting was provided by fluorescent lights which were on 24 hours per day.

Table A7_5_3_1_2-5: Cumulative mortalities and toxic symptoms observed in the bobwhite quail dietary test

Nominal dietary concentration [mg a.s./kg diet]	Dead	Dosed	Exhibiting toxic signs	Observations
Control 1	0	10	0	None
Control 2	0	10	0	None
312	0	10	0	None
625	0	10	0	None
1250	0	10	0	None
2500	1	10	0	None
5000	4	10	0	None

Table A7_5_3_1_2-6: Mean body weight data for bobwhite quails in the Triclosan dietary LC_{50} test

Nominal dietary concentration [mg a.s./kg diet]	Mean body weight [g]			Feed consumption [g/bird/day]	
	Day 0	Day +5	Day +8	Days 1-5	Days 6-8
Control 1	23	38	48	5	7
Control 2	23	34	44	5	6
312	23	37	47	5	7
625	23	37	46	5	7
1250	23	37	46	5	7
2500	23	36	46	5	7
5000	23	29	38	3	6

Table A7_5_3_1_2-7: Validity criteria for short-term avian toxicity test according to OECD Guideline 205

	Fulfilled	Not fulfilled
Mortality of control animals < 10 %	X	
Test substance concentration > 80 % of nominal concentration throughout the dosing period	X	
Lowest treatment level causing no compound-related mortality or other observable toxic effects	X	

Section A8 — MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

Subsection (Annex point)		In the following data from the Material Safety Data Sheet of DCPP is provided.	Official use only
8.1 (IIA, VIII 8.1)		**Recommended methods and precautions concerning handling, use, storage, transport or fire**	
8.1.0	**Methods and precautions concerning placing on the market**	Please refer to the information given below.	
8.1.1	**Methods and precautions concerning handling and use of the active substance**	Personal protection: Dust mask, protective gloves, safety glasses with side-shields, working clothes and closed footwear. Handle and open container with care. Avoid dust formation and ignition sources. Ensure good local exhaust ventilation. Do not eat, drink or smoke at the workplace. Close containers immediately after use. Avoid creating dusty conditions. Risk of explosion if an air-dust mixture forms. Inert container or use a system otherwise designed to prevent or contain an explosion-seek expert advice. (Reference: Anonymous, 2007)	x
8.1.2	**Methods and precautions concerning storage of the active substance**	Keep away from food and drink. Keep only in the original container. Keep container tightly closed. Avoid dust formation and ignition sources. Ensure good local exhaust ventilation. Do not eat, drink or smoke at the workplace. Keep in a dry, cool place. Protect from temperatures above 40 °C. (Reference: Anonymous, 2007) The active substance DCPP as manufactured is not packaged as such. Only the 30% formulation with the trade name [redacted] is placed on the market and imported by Ciba to Germany. Standard packaging item for [redacted] Polyethylene canister, 60 litre. Judged from the experience in use since many years [redacted] is not reactive towards the above mentioned container material. (Reference: [redacted] 2007a and 2007b)	x x
8.1.3	**Methods and precautions concerning transport of the active substance**	UN No.: 3077 (Reference: Anonymous, 2007)	x
8.1.4	**Methods and precautions concerning fire of the active substance**	Suitable extinguishing media: Water spray, foam, dry powder, carbon dioxide. Unsuitable extinguishing media for safety reasons: Water jet. Special protective equipment: Full protective clothing. Wear self-contained breathing apparatus. (Reference: Anonymous, 2007)	
8.2 (IIA, VIII 8.2)		**In case of fire, nature of reaction products, combustion gases, etc.** Carbon oxides, hydrogen chloride (Reference: Anonymous, 2007)	x

Section A8 MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

8.3 (IIA, VIII 8.3)		**Emergency measures in case of an accident**	
8.3.1	**Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment, if available**	If inhaled: Move to fresh air. Seek medical attention if you feel unwell or if exposure prolonged. In case of irritation of the respiratory system or mucous membranes, seek medical attention. On skin contact: Wash with plenty of soap and water. Do not use organic solvents. Get medical attention if irritation occurs. On contact with eyes: Rinse immediately with plenty of water for at least10 minutes taking care to wash under the eyelids. If irritation persists, seek medical attention. On ingestion: Rinse mouth immediately and then drink plenty of water, seek medical attention. Notes to physician: Treatment: Treat symptomatically. (Reference: Anonymous, 2007)	X
8.3.2	**Emergency measures to protect the environment**	Environmental precautions: Prevent entry into sewage systems, ground and surface waters. <u>Accidental release measures:</u> Personal precautions: Do not breathe vapours/dust. Sources of ignition should be kept well clear. Avoid contact with the skin, eyes and clothing. Methods for cleaning-up or taking-up: Take up mechanically and collect in suitable container (adequately labelled) for disposal. Collect waste in suitable containers, which can be labelled and sealed. Avoid raising dust. (Reference: Anonymous, 2007)	
8.4 (IIA, VIII 8.4)		**Possibility of destruction or decontamination following release in or on the following: (a) air, (b) water, including drinking water, and (c) soil**	
8.4.1	**Possibility of destruction or decontamination following release in the air**	The active substance DCPP is a solid with a very low vapour pressure. A contamination of the environmental compartment air is therefore unlikely after the release of DCPP into the environment due to an accidental misuse.	
8.4.2	**Possibility of destruction or decontamination following release in water, including drinking water**	From water or drinking water, DCPP should be removed according to common standard drinking water treatment procedures, for example by means of adsorptive material like activated charcoal filter.	
8.4.3	**Possibility of destruction or decontamination following release in or on soil**	Methods for cleaning-up or taking-up: Take up mechanically and collect in suitable container (adequately labelled) for disposal. Collect waste in suitable containers, which can be labelled and sealed. Avoid raising dust. (Reference: Anonymous, 2007)	
8.5 (IIA, VIII 8.5)		**Procedures for waste management of the active substance for industry or professional users**	

Section A8 — MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

8.5.1	**Possibility of re-use or recycling (IIA, VIII 8.5.1)**	Product disposal: Residual chemical should be disposed of by incineration or by other modes of disposal in compliance with local legislation. Contaminated packaging: Contaminated packaging should be emptied as far as possible and disposed of in the same manner as the substance/product. Dispose of in accordance with national, state and local regulations. Clean packaging material should be subjected to waste management schemes (recovery recycling, reuse) according to local legislation. (Reference: Anonymous, 2007)	x
8.5.2	**Possibility of neutralisation of effects (IIA, VIII 8.5.2)**	Please refer to the disposal considerations given above.	
8.5.3	**Conditions for controlled discharge including leachate qualities on disposal (IIA, VIII 8.5.3)**	Please refer to the disposal considerations given above.	x
8.5.4	**Conditions for controlled incineration (IIA, VIII 8.5.4)**	Please refer to the disposal considerations given above.	x
8.6 (IIA, VIII 8.6)		**Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms**	
		No undesirable or unintended side-effects on beneficial or other non-target organisms were observed.	x
8.7 (IIIA, VIII 1)		**Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances**	
		Organohalogen compounds are covered by List I of the Annex to Directive 80/68/EEC.	
		Biocides and their derivatives are covered by List II of the Annex to Directive 80/68/EEC.	x

Section A8 MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2012
Evaluation of applicant's justification	**8.1.1 Methods and precautions concerning handling and use of the active substance** This corresponds to Study A8. **8.1.2 Methods and precautions concerning storage of the active substance** This corresponds to Study A8 and Studies A 3.17/01 and A 3.17/02. **8.1.3 Methods and precautions concerning transport of the active substance** UN Number: This information was not reviewed, as under this point only "… transport must take into account any surface which could directly or indirectly come in contact with the product" is requested. This corresponds to Study A8. **8.2 In case of fire, nature of reaction products, combustion gases, etc.** This corresponds to Study A8. **8.5.1 Possibility of re-use or recycling** This corresponds to Study A8. **8.3.1 Specific treatment in case of an accident, e.g. first-aid measures, antidotes, medical treatment, if available** See P-Phrases in Doc II-A 1.5. **8.5.3 Conditions for controlled discharge including leachate qualities on disposal** *Add:* Diluted aqueous solutions (e.g. leachate) should be treated in a biological waste water treatment plant or by filtration through activated carbon. Direct discharge of DCPP containing solutions or suspensions into environmental waters must be avoided. **8.5.4 Conditions for controlled incineration** As DCPP is an organohalogen and dioxins and furans might be formed during incineration, appropriate burning conditions have to be guaranteed. **8.6 Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms** *Delete:* No undesirable or unintended side-effects on beneficial or other non-target organisms were observed. *Add:* DCPP is highly toxic to aquatic organisms, particularly to algae. From read across studies to the structural analogue triclosan considerable toxicity was observed to terrestric organisms, particularly to plants. The bioaccumulation potential was determined to be rather low. Effects on rodents and birds (read across with the structural analogue triclosan) can be considered rather low (see Doc II-A, chapter 4 for details). **8.7. Identification of any substances falling within the scope of List I or List II of the Annex to Directive 80/68/EEC on the protection of ground water against pollution caused by certain dangerous substances** *Add*: "not appearing in list I" after term "Biocides and their derivatives". Directive 80/68/EEC is repealed by the Water Framework Directive 2000/60/EC by 21.12.2012

Section A8 MEASURES NECESSARY TO PROTECT MAN, ANIMALS AND THE ENVIRONMENT

Conclusion	Agree with applicant´s version
Remarks	-

Section A9 Classification and Labelling

Annex Point IIA, IX

Current classification / labelling according to Directive 1967/548/EEC:	In the following the classification / labelling of DCPP is provided.	
Hazard symbol:	Xi; N	
Indication of danger:	Irritant Dangerous for the environment	
Risk phrases:	R41:	Risk of serious damage to eyes.
	R50/53:	Very toxic to aquatic organisms
Safety phrases:	S26:	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
	S39:	Wear eye/face protection.
	S60:	This material and its container must be disposed of as hazardous waste.
	S61:	Avoid release to the environment. Refer to special instructions/Safety data sheets.
Proposed classification / labelling according to Directive 1967/548/EEC:	Please refer to current classification and labelling.	
ustification:	Concerning the physico-chemical properties, the active substance DCPP does not fulfil the criteria for a classification according to Council Directive 67/548/EEC. Therefore no labelling is required for physico-chemical hazards. DCPP presents risk of serious damage to eyes and is very toxic to aquatic organisms. With regard to its toxicological and ecotoxicological properties, the active substance is classified as irritant and dangerous for the environment and has to be labelled with the hazard symbols Xi and N and the R-phrases R41-50.	

Section A9 Classification and Labelling

Annex Point IIA, IX

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	October 2012
Evaluation of applicant's proposal	
Conclusion	Proposed classification according to Reg. (EC) No 1272/2008 and Reg. (EU) No 286/2011: see Doc. II-A 1.5
Remarks	