Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA XII2.1

Official use only

		1 REFERENCE
1.1	**Reference**	[redacted] 1997, Aerobic Degradation of Dichlofluanid in Water-Sediment. [redacted]
1.2	**Data protection**	Yes
1.2.1	Data owner	[redacted]
1.2.2	Companies with letter of access	[redacted]
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, German BBA Guideline Part IV, 5-1 (December 1990)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	a) [Phenyl-UL-^{14}C] dichlofluanid b) non-active standard substance (dichlofluanid)
3.1.1	Lot/Batch number	a) [Phenyl-UL-^{14}C] dichlofluanid: Batch 31/1 b) non-active standard substance (dichlofluanid): Batch: 890524ELB01
3.1.2	Specification	a) specific radioactivity was 3.76 MBq/mg [redacted] b) see purity [redacted]
3.1.3	Purity	a) [redacted]adiochemical purity b) [redacted]urity
3.1.4	Further relevant properties	No problems related to abiotic stability or volatility are expected from the data available
3.1.5	Composition of Product	-
3.1.6	TS inhibitory to micro-organisms	Not to be expected because of the favourable results of the respiration inhibition tests in soil and sewage sludge
3.1.7	Specific chemical analysis	a) radiochemical purity: HPLC, radioactivity detector and TLC, scan b) chemical purity: HPLC, UV detector
3.2	**Reference substance**	No
3.2.1	Initial concentration of reference substance	-

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA XII2.1

3.3	**Testing procedure**		
3.3.1	Inoculum / test species	The water/sediment samples were taken from an artificially dammed pond (Hönniger Weiher, Wipperfürth, Germany) and a fenced-in fishing pond (Angler Weiher, Leverkusen, Germany)	
3.3.2	Test system	see table A7_1_2_2_2-2 In order to determine the exact DT-50 values two experiments were performed: a) Experiment I: performed only with the supernatant water (in 1 litre Erlenmeyer flask) b) Experiment II: carried out with water and sediment to confirm the results obtained with supernatant water only (500 ml microecosystem)	
3.3.3	Test conditions	see table A7_1_2_2_2-2	
3.3.4	Method of preparation of test solution	a) Experiment I: the test substance used was pure radio-labelled dichlofluanid. The radioactive compound was dissolved in 4.5 ml acetonitrile (Application solution I) and the radioactivity measured by liquid scintillation. A total of 450 µl (= 1,148.940 kBq) of Application solution I was applied to the vessels (= 0.306 mg a.i./500 ml water). b) Experiment II: the test substance used was a mixture of radio-labelled and unlabelled dichlofluanid. A total of 86 µl (11.2 mg dichlofluanid diluted in 1120 µl acetonitrile) was pipetted into a vessel and the solvent was evaporated. Application solution I (2500 µl) was added and radioactivity was determined (Application solution II). 300 µl (= 771.261 kBq) of Application solution II was applied to the vessels (= 0.308 mg/500 ml water + sediment)	X
3.3.5	Initial TS concentration	The amount of dichlofluanid applied to the water sediment systems was 0.60 mg/l. The maximum application rate in agriculture is up to 2.5 kg/ha, this amount corresponds to 0.83 mg/l (based on water depth of 30 cm). Since 0.83 mg/l is higher than 50% of the water solubility of dichlofluanid, this concentration was not used.	
3.3.6	Duration of test	up to 7 days	
3.3.7	Analytical parameter (methods)	Thin-Layer Chromatography: silica gel plates and RP-18 plates with different solvents methods for visualisation: autoradiography (radiolabelled compounds), UV lamp (unlabelled compounds). Spectroscopic analysis of the test substance and DMSA: GC-MS (INCOS XL instrument by Finnigan with Varian gas chromatograph) Radioactivity measurement of volatile compounds: a) Sorption on polyurethane foam plugs, extraction with ethyl acetate, which was measured by liquid scintillation. b) Sorption on sodium carbonate and release of CO_2 (after acidification) in a scintillation cocktail. Radioactivity measurement of solid samples (e.g. sediment): pre-treatment by e.g. drying and milling, then combustion and analysing radiolabelled CO_2	
3.3.8	Sampling	a) Experiment I: processing dates for the incubation vessels were 0.5 h, 2 h, 4 h, 7 h, 12 h, 17 h, 24 h, 3 days and 7 days	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA XII2.1

		b) Experiment II: processing dates for the incubation vessels were 1.5 h, 3.5 h, 4 h and 7 h	
3.3.9	Intermediates/ degradation products	Spectroscopic analysis of the test substance: GC-MS (INCOS XL instrument by Finnigan with Varian gas chromatograph)	
3.3.10	Nitrate/nitrite measurement	n.a.	
3.3.11	Controls	no control vessels	
3.3.12	Statistics	-	
		4 RESULTS	
4.1	**Degradation of test substance**		
4.1.1	Graph	Provided in the report	
4.1.2	Degradation	See tables A7_1_2_2_2-3 and A7_1_2_2_2-4	
4.1.3	Other observations	-	
4.1.4	Degradation of TS in abiotic control	Not relevant, because no hydrolytic degradation can be expected from the data, light induced degradation was excluded by running the experiment in the dark.	X
4.1.5	Degradation of reference substance	n.a.	
4.1.6	Intermediates/ degradation products	DMSA (dimethylaminosulfanilide); no further metabolite exceeded the 10% mark, DMSA degraded to CO_2	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The degradation and metabolism behaviour of [phenyl-UL-^{14}C]dichlofluanid was investigated in two experiments. With experiment I the degradation of dichlofluanid was investigated in two aquatic model ecosystems consisting of surface water only; experiment II was performed with water and sediment portion. Two different water/sediment systems were investigated according to BBA guideline IV, 5-1 (December 1990). Material balances were performed using radioactivity measurements of all test components.	
5.2	**Results and discussion**	The calculated DT-50 values (disappearance time of 50%) for dichlofluanid in the supernatant water of the two water-sediment systems were 1.1 and 2.7 hours. These values are relevant for natural surface water bodies. The DT-50 values for the total system of water and sediment were 1.2 and 3.0 hours (Experiment II) and for the supernatant water without sediment 1.5 and 3.0 hours (Experiment I). Dichlofluanid was as fast degraded in water-sediment systems as in systems consisting of water only.	X
5.3	**Conclusion**	The results in this test show that dichlofluanid was very rapidly degraded in aerobic aquatic systems to DMSA (dimethylaminosulfanilide). There was no further metabolite approaching or exceeding the 10% mark within the incubation time.	X

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA XII2.1

		Dichlofluanid does not constitute a lasting potential to contaminate surface water or sediment. The study is well documented and reported. A complete material balance was performed at all samplings by radioactive analysis. The parameters from two blank water sediment systems show no deviations from the fortified systems.
5.3.1	Reliability	Reliability indicator: 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	30/11/2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	
Remarks	

Section A7.1.2.2.2 Water/sediment degradation study

Annex Point IIIA XII2.1

	COMMENTS FROM ...
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	

Table A7_1_2_2_2-1: Properties of the Natural Water Sediment Systems

System	Property	Hönniger Weiher	Angler Weiher
Supernatant water	Hardness [dH°]	4.1	12.1
	N(total) [mg/l]	2.0	2.4
	P(total) [mg/l]	0.6	0.5
	TOC	1.6	1.7
	DOC	1.6	1.7
Sediment	Sediment (0-10 cm)	loam	sandy loam
	Texture analysis (USDA); sand/silt clay [%]	38.5/47.1/14.4	69.0/21.8/9.2
	pH (in water/in 0,01 M CaCl2)	5.8/5.4	7.3/6.7
	CEC [meq/100 g dry sediment]	10	< 1
	Organic carbon/humus [mg/100 g dry sediment]	4070/7000	2310/3970
	N(total) [mg/100 g dry sediment]	310	180
	P(total) [mg/100 g dry sediment]	89.4	37.4

Table A7_1_2_2_2-2: Test system and Test conditions

Criteria	Details
Culturing apparatus	a) Experiment I: 500 ml samples of supernatant water were pored into 1 litre Erlenmeyer flasks); b) Experiment II: carried out with water and sediment in microecosystems; the glass vessels containing 310 ml water and 190 ml sediment (to reach a sediment height of 2.5 cm); total volume: 500 ml each. Dry weight of sediment in flask: 128.6/163.0 g (Hönniger Weiher/Angler Weiher)
Number of culture flasks/concentration	a) Experiment I: total of 4 batches (two water systems, each replicates A and B) b) Experiment II: total of 6 batches (two water systems, partly two replicates A and B)
Aeration device	Not applied
Measuring equipment	In the supernatant water measurements of the oxygen content, pH-value and redox potential were performed; the redox potential of the sediment was also determined during the experiments.
Composition of medium	see table A7_1_2_2_2_1
Additional substrate	No
Pre-incubation of the test systems	yes, 22 days
Test temperature	20.5 ± 0.5 °C
pH at the begin/end of the study	Experiment I: Hönniger Weiher: 7.4/7.7, Angler Weiher: 8.0/8.1 Experiment II: Hönniger Weiher: 7.5/7.6, Angler Weiher: 8.1/8.1
Oxygen content at the begin of the study (in % of maximum oxygen content: at 20°C: 8.84 mg O_2/l)	Experiment I: Hönniger Weiher: 94/90%, Angler Weiher: 86/90% Experiment II: Hönniger Weiher: 95/91%, Angler Weiher: 88/88%
Aeration of dilution water	No
Suspended solids concentration	not determined
Other relevant criteria	a) the test was conducted in the dark, b) the water phase was slowly stirred by a magnetic stirrer to maintain oxygen uptake

Table A7_1_2_2_2-3: Distribution of dichlofluanid and DMSA [% of applied radioactivity] in natural water after application of 0.60 mg/l [phenyl-UL-^{14}C]dichlofluanid (Experiment I)

	Incubation time						
	0 min	0.5 h	2 h	7 h	1 d	3 d	7 d
Hönniger Weiher							
water after extraction	0.1	0.1-0.2	0.1-0.2	0.3	0.3	0.3	0.4-0.5
Dichlofluanid (dichloromethan extr.)	99.4	90.8	67.3-70.6	14.8-16.3	< 0.1	< 0.1	n.d.
DMSA (dichloromethan extr.)	0.5	7.9-9.1	27.4-31.5	79.9-84.3	98.3-98.5	99.2-100	97.6-98.3
Unknown(s)	n.d.	n.d.	n.d.	n.d.	0.1	n.d.	n.d.
Angler Weiher							
water after extraction	0.1	0.1-0.2	0.2-0.3	0.3-0.4	0.3-0.4	0.3	0.3-0.4
Dichlofluanid (dichloromethan extr.)	99.2	79.2-80.7	41.6-42.5	1.8-2.1	< 0.1	< 0.1	n.d.
DMSA (dichloromethan extr.)	0.7	19.1	56.2-56.9	96.9-98.5	96.9-97.0	99.2-99.7	98.2-99.3
Unknown(s)	n.d.	n.d.	n.d.	n.d.	0.1-< 0.1	n.d.	n.d.

Table A7_1_2_2_2-4: Distribution of radioactivity [% of applied] in two water/sediment systems after application of 0.60 mg/l [phenyl-UL-^{14}C]dichlofluanid (Experiment II)

		Hönniger Weiher incubation time			Angler Weiher incubation time		
		0 min	3.5 h	7 h	0 min	1.5 h	4 h
supernatant water	**total**	**100.0**	**79.9-82.8**	**83.8**	**100.0**	**81.5-83.4**	**83.7**
	water after extraction	0.1	0.8	1.3	0.1	1.1-1.3	1.1
	Dichlofluanid (dichloromethan extract)	99.4	43.5-50.7	2.2	99.2	30.9-44.9	9.1
	DMSA (dichloromethan extract)	0.5	28.4-38.5	80.3	0.7	35.5-51.2	73.4
sediment	**total**	**0.0**	**13.7-14.3**	**13.0**	**0.0**	**12.2-13.9**	**12.0**
	Dichlofluanid (organic sediment extract)	0.0	5.7-6.9	2.1	0.0	4.0-6.9	0.3
	DMSA (organic sediment extract)	0.0	7.1-7.7	10.6	0.0	6.9-8.1	11.4
	aqueous sediment extract	0.1	0.1	0.1	< 0.1	< 0.1	0.1
	bound residues	0.0	0.2	0.2	0.0	0.1	0.2
Sum of individual	**Dichlofluanid**	**99.4**	**49.2-57.6**	**4.3**	**99.2**	**34.9-51.8**	**9.4**
	DMSA	**0.5**	**35.5-46.2**	**90.9**	**0.7**	**42.4-59.3**	**84.4**

Section A7.1.3 Adsorption / Desorption screening test of

Annex Point IIA7.7 **DIMETHYLAMINOSULFANILID (DMSA)**

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2001, Estimation of the Adsorption Coefficient (K_{OC}) of DMSA on Soil using High Performance Liquid Chromatography (HPLC) [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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 / authorisation	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes, OECD Guideline for the Testing of Chemicals, Proposal for a new Guideline 121, "Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)"(2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)	
3.1.1	Lot/Batch number	M00195 [redacted]	
3.1.2	Specification		
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	-	X
3.1.5	Method of analysis	HPLC, fitted with a pulse-free pump and a suitable detection device	
3.2	**Degradation products**	DMSA is the metabolite of the active substance dichlofluanid.	
3.3	**Reference substance**	Yes, thirteen reference substances were used to determine an average capacity factor k`: Acetanilide, Aniline, Atrazine, Cyfluthrin, N,N-dimethylbenz-amide, DMST, Fenthion, Isoproturon, Linuron, Methiocarb, Phenan-trene, Pyrazophos and Triadimenol. Sodium nitrate was used to determine the HPLC dead time (t_0).	X
3.3.1	Method of analysis for reference	HPLC	

	substance		
3.4	**Testing procedure**		X
3.4.1	Test system	HPLC (HP 1090) is performed on analytical columns packed with a commercially available cyanopropyl solid phase containing lipophilic and polar moieties (Zorbax CN, 5 µm, length = 250 mm, i.d. = 4.6 mm). As mobile phase methanol/0.01 M citrate-buffer pH 6.0 (55/45, v/v) was used. As a result of partitioning between mobile and stationary phases the test substance is retarded. The dual composition of the stationary phase having polar and non-polar sites allows for interaction of polar and non-polar groups of a molecule in a similar way as is the case for organic matter in soil. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established.	
3.4.2	Test solution and Test conditions	According to the guideline, the maximum concentration of the test substance should not exceed ½ the solubility in the solvent. Therefore the measurements were carried out at concentrations of approx. 5 mg/l. Stock solution: 9.62 mg DMSA were weighed into a 10 ml volumetric flask and diluted to volume with methanol. Standard solution: 0.1 ml of the stock solution was transferred into a 20 ml volumetric flask and diluted to volume with the mobile phase methanol/citrate buffer pH 6.0. The flask was shaken and ultrasonicated for one minute to dissolve the substance. HPLC parameters: Oven temperature: 40 °C, Injection volume: 250 µl, Flow rate: 1.5 ml/min and Run time: 30 min.	
3.5	**Calculations**	**K_d:** Distribution coefficient is defined as the ratio of equilibrium concentrations C of a dissolved test substance in a two phase system consisting of a sorbent (soil or sewage sludge) and an aqueous phase. It can be dimensionless or have the dimension mg/l. **K_{OC}:** Distribution coefficient (K_d) or Freundlich adsorption coefficient (K_f) normalised to the organic carbon content (f_{OC}) of a sorbent. Depending on the dimensions of K_d and K_f, K_{OC} can be dimensionless or have the dimensions ml/g or µg/g organic matter. Using the HPLC estimation method the adsorption coefficient (K_{OC}) is deduced from the capacity factor (k`) using a calibration plot of log k` vs. log K_{OC} of the selected reference substances. K_{OC} is an approximate indicator for the extent of adsorption between a substance and the sorbent and allows comparison to be made between different chemicals. **k`:** Capacity factor = $\frac{t_R - t_0}{t_0}$ t_R = HPLC retention time of test and reference substance (min) t_0 = HPLC dead time (min)	X

		log K_{OC}: log K_{OC} = Slope • log k` + intercept; Slope and intercept derived from the linear regression of the reference standards using K_{OC}.	
		4 RESULTS	X
4.1	**Measurements**	HPLC retention time data for the reference substances and the test substance dimethylaminosulfanilid (DMSA) are given in table A7.1.3.1_1. The dead time (t_0) was determined to be 1.536 minutes using sodium nitrate. Variability of the retention times from repetitive injections was low, confirming HPLC system stability throughout the analysis period.	X
4.2	**Calculations**	Calculated adsorption parameter for the reference substances and the test substance dimethylaminosulfanilid (DMSA) are given in table A7.1.3.1_1.	
4.3	**Degradation product(s)**	DMSA is the metabolite of the active substance dichlofluanid.	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The adsorption coefficient K_{OC} of dimethylaminosulfanilid (DMSA) on soil was estimated using High Performance Liquid Chromatography (HPLC). The test was performed according to the OECD Guideline for the testing of chemicals, Proposal for a new Guideline 121, "Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)" (2001) in order to determine the mobility of DMSA in soil. Thirteen reference standards of known K_{OC} were analysed on a HPLC system to determine an average capacity factor k`. Sodium nitrate was used to determine the HPLC system dead time ($t_0$). A regression line was plotted with the determined k` values and the known K_{OC} values (log k` vs. log K_{OC}). The study shows no significant deviations from the test guideline.	
5.2	**Results and discussion**	Dimethylaminosulfanilid (DMSA) was analysed on the same HPLC system during the same sample sequence as the reference substances, and average k` values were determined. The $K_{OC}$ value for DMSA was estimated by interpolation from the reference substance regression line.  The linear regression of measured k` values against literature K_{OC} values yielded a line with a slope of 4.41, an intercept of 2.46 and a correlation coefficient R^2 of 0.893. The estimated K_{OC} value for DMSA is 53.	
5.3	**Conclusion**	Based on classification of Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham/UK, 83-86, 1973) and Verdam et al. (RIVM, Rapport No. 728473001, NL, 1988) for the estimation of the mobility of plant protectants in soil based on K_d and/or K_{OC}-values, dimethylaminosulfanilid (DMSA) is to be classified as an intermediate mobile substance.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.1.3 **Adsorption / Desorption screening test of**

Annex Point IIA7.7 **DIMETHYLAMINOSULFANILID (DMSA)**

Section A7.1.3 Adsorption / Desorption screening test of

Annex Point IIA7.7 **DIMETHYLAMINOSULFANILID (DMSA)**

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2/12/2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion **Reliability** **Acceptability** **Remarks**	[redacted]
[redacted]	[redacted]

Section A7.1.3 Adsorption / Desorption screening test of

Annex Point IIA7.7 **DIMETHYLAMINOSULFANILID (DMSA)**

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	

Table A7.1.3.1_1 HPLC Retention Time Data and K_{OC} Calculations

Substance	Mean Retention Time [min]	Mean Dead Time [min]	Mean k´	Mean log k´	Mean K_{OC}	Mean log K_{OC}
Sodium nitrate	-	1.536	-	-	-	-
Acetanilide	2.485	1.536	0.62	-0.21	17.8	1.25
N,N-dimethyl-benzamide	2.643	1.536	0.72	-0.14	33.1	1.52
Atrazine	2.746	1.536	0.79	-0.10	64.6	1.81
Isoproturon	2.966	1.536	0.93	-0.03	72.4	1.86
Aniline	2.485	1.536	0.62	-0.21	117	2.07
Triadimenol	3.044	1.536	0.98	-0.01	251	2.40
Linuron	3.323	1.536	1.16	0.07	389	2.59
Methiocarb	3.012	1.536	0.96	-0.02	1,259	3.10
Fenthion	4.013	1.536	1.61	0.21	2,042	3.31
Pyrazophos	4.010	1.536	1.61	0.21	4,467	3.65
Phenantrene	4.600	1.536	2.00	0.30	12,303	4.09
Cyfluthrin	7.496	1.536	3.88	0.59	64,300	4.81
DMST	2.693	1.536	0.76	-0.12	76.25	1.88
DMSA	**2.580**	**1.536**	**0.679**	**-0.168**	**52.95**	**1.724**

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 2001, Estimation of the Adsorption Coefficient (Koc) of Dichlofluanid on Soil using High Performance Liquid Chromatography. [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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 121 (Proposal for a New Guideline 121, January 2001)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dichlofluanid	
3.1.1	Lot/Batch number	890524ELB01[redacted]	
3.1.2	Specification	As given in section 2 of the dossier	
3.1.3	Purity	[redacted]	
3.1.4	Further relevant properties	-	X
3.1.5	Method of analysis	HPLC, fitted with a pulse-free pump and a suitable detection device according to OECD Guideline 121	
3.2	**Degradation products**	Degradation products tested: Yes; Main metabolite: dimethylaminosulfanilide (DMSA)	
3.2.1	Method of analysis for degradation products	HPLC	
3.3	**Reference substance**	Yes, 13 reference compounds were used to determine an average capacity factor k': acetanilide, N,N-dimethylbenzamide, Atrazine, Isoproturon, Aniline, Triadimenol, Linuron, Methiocarb, Fenthion, Pyrazophos, Phenantrene, Cyfluthrin and dimethylaminosulfanilide (DMSA). Sodium nitrate was used to determine the HPLC dead time (t_0).	X
3.3.1	Method of analysis for reference substance	HPLC	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

3.4	**Testing procedure**		X
3.4.1	Test system	HPLC (HP 1090 with DAD detector) is performed on analytical columns (Zorbax CN, length 250 mm, i.d. 4.6 mm) packed with a commercially available cyanopropyl solid phase containing lipophilic and polar moieties. As mobile phase methanol/0.01 M citrate-buffer pH 6.0 (55/45, v/v) was used. As a result of partitioning between mobile and stationary phases the test substance is retarded. The dual composition of the stationary phase having polar and non-polar sites allows for interaction of a molecule in the similar way as in the case for organic matter in soil. This enables the relationship between the retention time on the column and the adsorption coefficient on organic matter to be established.	
3.4.2	Test solution and Test conditions	According to guideline, maximum concentration of the test substance should not exceed 50% of the solubility in the solvent. Therefore the measurements were carried out at concentrations of approx. 5 mg/l. Stock solution: 11.68 mg dichlofluanid was weighed into a 10-ml volumetric flask and diluted to volume with methanol. Standard solution: 0.1 ml of stock solution was transferred into a 20-ml volumetric flask and diluted to volume with the mobile phase methanol/citrate buffer pH 6.0. HPLC parameters: Oven temperature 40 °C, Injection volume 250 µl, Flow rate 1.5 ml/min, run time 30 min.	
3.5	**Calculations**	**Kd:** Distribution coefficient is defined as the ratio of equilibration concentrations C of a dissolved test substance in a two phase system consisting of a sorbent (soil or sewage sludge) and an aqueous phase. It can be dimensionless or have the dimension ml/g. **Koc:** Distribution coefficient (Kd) or Freundlich adsorption coeffcient (Kf) normalised to the organic carbon content (foc) of a sorbent. Depending on the dimensions of Kd and Kf, Koc can be dimensionless or have the dimensions ml/g or µg/g organic matter, respectively. Using the HPLC estimation method Koc is deduced from the capacity factor (k') using a calibration plot of log k' versus log Koc of the selected reference compounds. Koc is an indicator for the extension of adsorption between a substance and the sorbent and allows comparisons to be made between different chemicals. **k':** Capacity factor = $(t_R - t_0)/t_0$; t_R = HPLC retention time of test and reference substances (min); t_0 = HPLC dead time (min). **log Koc** = Slope x log k' + Intercept; Slope and intercept derived from the linear regression of the reference standards using Koc.	X
		4 RESULTS	X
4.1	**Measurements**	HPLC retention time data for the reference compounds and dichlofluanid are given in table A7.1.3_1. The dead time t_0 was determined to be 1.536 min using sodium nitrate. Variability of the retention times from repetitive injections was low, confirming HPLC system stability throughout the analysis period.	X

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

4.2	**Calculations**	Calculated adsorption parameter for the reference compounds and dichlofluanid are given in table A7.1.3.1_1.	
4.3	**Degradation product(s)**	DMSA as main metabolite was investigated as reference substance. For results see table A7.1.3.1_1.	X
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The adsorption koefficient Koc of dichlofluanid on soil was estimated using High Performance Liquid Chromatography (HPLC). The test was performed according to OECD Guideline 121 (Proposal for New Guideline, 2001). Thirteen reference standards of known Koc were analysed on a HPLC system to determine an average capacity factor k'. Sodium nitrate was used to determine the HPLC system dead time (t_0). A regression line was plotted with the determined k' values and the known Koc values (log k' versus log Koc).	
5.2	**Results and discussion**	Dichlofluanid was analysed on the same HPLC system during the same sample sequence as the reference substances and an average k' value of 1.415 was determined. The Koc value for dichlofluanid was estimated by interpolation from the reference substance regression line. The linear regression of measured k' values against literature Koc values yielded a line with a slope of 4.41, an intercept of 2.46 and a correlation coefficient R^2 of 0.893. The estimated Koc value for dichlofluanid is 1344.	
5.3	**Conclusion**	Based on classifications of Briggs (Proc. 7th British Insecticide and Fungicide Conference, Nottingham, UK, 83-86, 1973) and Verdam et al. (RIVM Report No. 728473001, NL, 1988) for the estimation of the mobility of plant protectants in soil based on Kd and/or Koc-values, dichlofluanid is to be classified as an immobile substance.	
5.3.1	Reliability	1	
5.3.2	Deficiencies	No	

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	2/12/2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion **Reliability** **Acceptability** **Remarks**	[redacted]
	COMMENTS FROM ...
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*

Section A7.1.3 Adsorption / Desorption screening test

Annex Point IIA.7.7

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	

Table A7.1.3.1_1: HPLC retention time data and Koc calculations

Substance	Mean Retention Time [min]	MeanDead Time [min]	Mean k'	Mean log k'	Mean Koc	Mean log Koc
Sodium nitrate	-	1.536	-	-	-	-
Acetanilide	2.485	1.536	0.62	-0.21	17.8	1.25
N,N-dimethylbenzamide	2.643	1.536	0.72	-0.14	33.1	1.52
Atrazine	2.746	1.536	0.79	-0.10	64.6	1.81
Isoproturon	2.966	1.536	0.93	-0.03	72.4	1.86
Aniline	2.485	1.536	0.62	-0.21	117	2.07
Triadimenol	3.044	1.536	0.98	-0.01	251	2.40
Linuron	3.323	1.536	1.16	0.07	389	2.59
Methiocarb.	3.012	1.536	0.96	-0.02	1,259	3.10
Fenthion	4.013	1.536	1.61	0.21	2,042	3.31
Pyrazophos,	4.010	1.536	1.61	0.21	4,467	3.65
Phenantrene	4.600	1.536	2.00	0.30	12,303	4.09
Cyfluthrin	7.7.496	1.536	3.88	0.59	64,300	4.81
Dimethylaminosulfanilide (DMSA)	2.693	1.536	0.76	-0.12	76.25	1.88
Dichlofluanid	**3.710**	**1.536**	**1.415**	**0.1507**	**1,344**	**3.13**

Section A7.2.1 Aerobic degradation in soil

Annex Point: IIIA XII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1988, Metabolism of [benzene-ring-UL-14C] dichlofluanid (Euparen®) in soil under aerobic conditions [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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**	EPA Pesticide Assessment Guidelines § 162-1, October 1982	
2.2	**GLP**	No, GLP requirements of 40 DFR Part 160 do not apply to the study described.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) [benzene ring-UL-^{14}C] dichlofluanid b) non-active standard substance (dichlofluanid)	
3.1.1	Lot/Batch number	No lot or batch no. mentioned	
3.1.2	Specification	a) specific radioactivity was 1246.9 kBq/mg, [redacted] b) as given in section 2 of dossier [redacted]	
3.1.3	Purity	a) [redacted] radiochemical purity b) [redacted] purity	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	Soil was extracted with methanol/water and dichlormethane. Extracts were pooled radioassayed by LSC and analysed with HPLC and TLC. Analysing of bound residues: the soil was treated with 0,5 M NaOH and extracted for 24 hours. After centrifugation the radioactivity in the sediment was determined by ashing (humin). To precipitate the humic acid fraction the supernatant was acidified with HCl to a pH of 2. The radioactivity of the supernatant (fulvic acid) and the sediment taken up in 0.5 M NaOH (humic acid) was determined. The quantification of the humic acid, fulvic acid and humin fraction was done with LSC. Verification of microbial activity was accomplished by monitoring the evolved $^{14}CO_2$ from 100 g soil. Seperate batches were available to detect	X

Section A7.2.1 Aerobic degradation in soil

Annex Point: IIIA XII 1.1

		the microbial biomass at the start of the test and at certain sampling points. CO_2 trapping solutions were radioassayed by LSC.	
3.2	**Reference substance**	Dichlofluanid, Dimethylaminosulfanilide (DMSA), Methylaminosulfanilide (KUE 8630B), Amino sulfoanilide (KUE 9079A), 4-Hydroxydimethylaminosulfoanilide (KUE 86630A and KUE 8630C) and Phenylamido sulfonic acid (K-salt) (THS 3245)	
3.2.1	Method of analysis for reference substance	Dichlofluanid, Dimethylaminosulfanilide and Methylaminosulfanilide were extracted with methanol and measured by GC-MS	
3.3	**Soil types**	Three soil types were used, see table A7_2_1-1	**X**
3.4	**Testing procedure**		
3.4.1	Test system	Incubation vessel for aerobic soil metabolism studies (according to J.P.E. Anderson: Soil Biol. Biochem., 10, p. 215-221 (1978)). Radioactive labelled dichlofluanid was dissolved in ethyl acetate and applied to 100 g soil screened to a particle size ≤ 2 mm via a subsample, resulting in a concentration of 10 mg/kg. Then incubated in glass flasks with CO_2-trap under aerobic conditions in the dark at 23 ± 2 °C. The flasks were sampled at day 1, 3, 8, 14, 30, 59, 97 181 (Variant 1a); at day 0, 1, 3, 8 (Variant 1b and 1c); at day 8, 30, 90, 181 (Variant 2); at day 0, 30, 61, 90 183 (Variant 3); at day 0, 30, 58, 97, 132, 181, 280 and 414 (Variant 4). In test variant 2 (with steril soil) the parent compound solution was dripped onto the sterile soil under sterile conditions.	
3.4.2	Test solution and Test conditions	A separate stock solution was prepared for each soil type. The radioactive labelled dichlofluanid was dissolved in ethyl acetate and mixed with unlabelled parent compound.	
		4 RESULTS	
4.1	**Aerobic soil metabolism**	See table A7_2_1-2	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA Guideline 162-1 was followed. The soil metabolism of [benzene ring-UL-14C] dichlofluanid under aerobic conditions was investigated in two sandy loam soils (soil 1 and 3) and a sand soil (soil 2). In a variation of the test, work was performed with sterile soil 2. The average concentration of dichlofluanid was 10 mg/kg soil.	
5.2	**Results and discussion**		
5.2.1	DT50 values	In biological active soils the half-life of dichlofluanid was less than one day (DT50 < 1 day). In the sterile soil after 90 days still 53.2% of dichlofluanid were present (DT50 > 90 days).	
5.2.2	Degradation	Dichlofluanid was rapidly degraded by biological active soils to	

Section A7.2.1 Aerobic degradation in soil

Annex Point: IIIA XII 1.1

	products (% of a.s.)	dimethylaminosulfanilide (DMSA). After 1 day 79.5-84.0% of the parent compound was degraded to DMSA. After 90 days the percentage of parent compound was less than 0.1% in the living soils. Beside dimethylaminosulfanilid (DMSA) a further metabolite could be identified as methylaminosulfanilide (KUE 8630B). This metabolite reached his highest concentration (8.2%) in soil 1 after 97 days.
5.2.3	Bound residues	The bound residues in the living soils after 30 days were at a level between 24.2% and 42.5%. At the end of the study 56% bound residues were found in soil 1 (after 181 d), 69.4 in soil 3 (after 183 d) and 75.7% in soil 4 (after 414 d), respectively. In the sterile variant (soil 2) only max. 4.8% of the applied radioactivity was found in the bound residues fraction (after 181 d). Dimethylaminosulfanilide and small quantities of methylamino-sulfanilide could be released from this residue after hydrochloric acid/acetone extraction.
5.2.4	CO_2 formation	The CO_2 formation in the biological active soils was 9.2% (soil 3) to 22.6% (soil 4) at the end of the experiments (183 and 414 days, respectively). Under sterile soil conditions a CO_2 formation of only 0.2% of applied radioactivity was detected after 181 days.
5.3	**Conclusion**	Dichlofluanid is rapidly degraded in biological active soils to dimethylaminosulfanilide (DMSA). Under such conditions the half-life of dichlofluanid is less than one day (DT50 < 1 day). In sterile soil the degradition of dichlofluanid is much slower (DT50 > 90 days).
5.3.1	Reliability	2
5.3.2	Deficiencies	Batch numbers of test compound not given

Section A7.2.1 Aerobic degradation in soil

Annex Point: IIIA XII 1.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24/11/2004
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
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	

Table A7_2_1-1: Classification and physico-chemical properties of soils used

	Soil 1	Soil 2	Soil 3
Name	Speyer II standard soil (= BBA soil 2.2)	Speyer I standard soil (= BBA soil 2.1)	Kansas
Location	Hanhofen, Germany	Jockgrim, Germany	Stanley Research Center, Kansas City, USA
Soil texture	sandy loam	sand	sandy loam
Sand [%]	80	87	67
Silt [%]	12	9	27
Clay [%]	8	4	6
Organic carbon [%]	2.6	0.8	1.3
pH (0.01 M $CaCl_2$)	7.1	5.4	5.2
Cation exchange capacity (MEQ/100 g at pH 8.2)			
Biomass at start of study [mg microbial C/kg dry weight soil]	340	90	243

Table A7_2_1-2: Degradation in soil under standard laboratory conditions

	Variant 1a: Soil 1 (living)	Variant 2: Soil 1 (sterile)	Variant 3: Soil 2 (living)	Variant 4 Soil 3 (living)
Dose [mg/kg soil]	10	10	10	10
Incubation [days]	181	181	183	414
Dichlofluanid [%]	< 0.1	49.7	< 0.1	< 0.1
DMSA [%]	17.8	46.1	8.5	1.4
KUE 8630B [%]	7.3	-	2.4	1.2
Not identified [%]	1.8	-	3.5	0.7
$^{14}CO_2$ [%]	10.9	0.2	9.2	22.6
Bound residues	56.0	4.8	69.4	75.7
a. Fulvic acid	22.7			
b. Humic acid	17.6			
c. Humin	11.9			
Total recovered radioactivity [%]	93.8	100.8	93.0	

Section A7.2.2.4. Anaerobic degradation in soil

Annex Point: IIIA XII 1.1

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1988, Metabolism of [ring-UL-14C] dichlofluanid (Euparen®) in soil under anaerobic conditions [redacted]	X
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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**	EPA Pesticide Assessment Guidelines § 162-2, October 1982	
2.2	**GLP**	No, GLP requirements of 40 DFR Part 160 do not apply to the study described.	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) [benzene ring-UL-^{14}C] dichlofluanid b) non-active standard substance (dichlofluanid)	
3.1.1	Lot/Batch number	No lot or batch no. mentioned	
3.1.2	Specification	a) specific radioactivity was 1246.9 kBq/mg [redacted] b) as given in section 2 of dossier [redacted]	
3.1.3	Purity	[redacted]adiochemical purity b[redacted]urity	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	Soil was extracted with one portion of methanol/water and two portions of methanol. Extracts were combined, pooled radioassayed by LSC and analysed with HPLC and TLC. Analysing of bound residues: the soil samples, which had been extracted with solvents, were dried, ground in a mill and ashed in an automatic sample oxidizer.	
3.2	**Reference substance**	Dichlofluanid, Dimethylaminosulfanilide (DMSA), Methylaminosulfanilide (KUE 8630B), Amino sulfoanilide (KUE 9079A), 4-Hydroxydimethylaminosulfoanilide (KUE 86630A and KUE 8630C) and Phenylamido sulfonic acid (K-salt) (THS 3245)	
3.2.1	Method of analysis for reference	Dichlofluanid, Dimethylaminosulfanilide and Methylaminosulfanilide were extracted with methanol and measured by GC-MS.	

Section A7.2.2.4. Anaerobic degradation in soil

Annex Point: IIIA XII 1.1

	substance		
3.3	**Soil types**	One soil was used, see table A7_2_2_4-1	
3.4	**Testing procedure**		
3.4.1	Test system	Radioactive labelled dichlofluanid was dissolved in ethyl acetate and applied to soil screened to a particle size ≤ 2 mm via a subsample, resulting in a concentration of 9 mg/kg. Variant A: Incubation vessels for anaerobic soil metabolism studies were used; anaerobic conditions from the start of experiment. The 100 g soil samples were mixed with 80 ml of distilled water gasified with N_2 so that a layer of approx. 2 cm deep stood above the soil surface. The conical flasks were then flushed out with nitrogen, closed and stored in the dark at 22 °C (± 2 °C). The flasks were sampled at day 30, day 61 and day 90; Variant B: For these samples, anaerobic degradation was preceded by a period of aerobic preincubation in vessels for aerobic soil metabolism studies (according to J.P.E. Anderson: Soil Biol. Biochem., 10, p. 215-221 (1978)). After 30 days aerobic preincubation, the soil samples switched to anaerobic conditions as described for variant A. The flasks were sampled at day 31 and 60.	
3.4.2	Test solution and Test conditions	Labelled and unlabelled dichlofluanid were dissolved and mixed; 100 µl of the stock solution contained 0.87 mg a.i. (321.374 kBq).	
		4 RESULTS	
4.1	**Aerobic soil metabolism**	See table A7_2_2_4-2	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	US EPA Guideline 162-2 was followed. The soil metabolism of [benzene ring-UL-14C] dichlofluanid under anaerobic conditions was investigated in a sandy loam soil. In a variation of the test, an aerobic preincubation of the samples was performed. The average concentration of dichlofluanid was 9 mg/kg soil	
5.2	**Results and discussion**		
5.2.1	DT50 values	not determined	
5.2.2	Degradation products (% of a.s.)	In anaerobic soils dichlofluanid was rapidly degraded to dimethyl-aminosulfanilide (DMSA). After 30 days 87.4-95.5% of the parent compound was degraded to DMSA and the percentage of parent compound was less than 0.1%. Small amounts of methylaminosulfanilide (KUE 8630B) were also detected (≤ 0.2%).	
5.2.3	Bound residues	The bound residues after 30 days were at a level between 6.8% and 11.9%. At the end of the study 10.6-11.1% bound residues were found (after 90 d). In the variant with aerobic preincubation the proportion of bounded	**X**

Section A7.2.2.4. Anaerobic degradation in soil

Annex Point: IIIA XII 1.1

		residues was distinctly lower than in the purely anaerobic systems (47.5-49.9% after 31 days.
5.2.4	CO_2 formation	The CO_2 formation in the biological active soils was very low (≤ 0.3%).
5.3	**Conclusion**	Under anaerobic conditions in soil dichlofluanid is rapidly degraded to dimethylaminosulfanilide (DMSA).
5.3.1	Reliability	2
5.3.2	Deficiencies	Batch numbers of test compound not given

Section A7.2.24 Anaerobic degradation in soil

Annex Point: IIIA XII 1.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	31/08/05
Materials and Methods	[redacted]
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
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	

Table A7_2_2-4-1: Classification and physico-chemical properties of the soil used

	Soil
Location	Stanley Research Center, Kansas City, USA
Soil texture	sandy loam
Sand [%]	67
Silt [%]	27
Clay [%]	6
Organic carbon [%]	4.6
pH (0.01 M $CaCl_2$)	5.2
Biomass at start of study [mg microbial C/kg dry weight soil]	268

Table A7_2_2_4-2: Degradation in soil under standard laboratory conditions

	Variant A: anaerobic degradation			Variant B anaerobic degradation with aerobic preincubation	
Dose [mg/kg soil]	9			9	
Incubation [days]	30	61	90	30/31(aerobic / anaerobic)	30/60(aerobic / anaerobic)
Dichlofluanid [%]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
DMSA [%]	87.4-95.5	80.4-89.1	88.1-88.3	35.2-35.6	28.3-28.8
KUE 8630B [%]	< 0.1-0.2	< 0.1	< 0.1	8.1	7.1-7.5
Not identified [%]	< 0.1-0.1	0.1	< 0.1	0.6-0.8	2.1
$^{14}CO_2$ (headspace + water) [%]	< 0.1	0.1-0.3	< 0.1-0.1	3.4-3.6	5.1-7.1
$^{14}CH_4$ [%]	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Bound residues	6.8-11.9	8.1-20.5	10.6-11.1	47.5-49.9	58.7-60.0
Total recovered radioactivity [%]	99.6-102.3	97.4-101.3	99.0-99.4	95.4-97.4	103.1-103.8

Section A7.2.3.2 Aged residues soil leaching study

Annex Point IIIA XII 1.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1985, Leaching characteristics of Dichlofluanid (Euparen®) aged in soil. [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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, Federal German Biological Agency for Agriculture and Forestry (BBA), Bulletin No. 37, 2nd edition, February 1980 and Draft for 3rd edition (1984).	
2.2	**GLP**	GLP requirements of 40 CFR Part 160 do not apply to the study described in this document	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) ($U^{14}C$)-phenyl-dichlofluanid b) non-active standard substance (dichlofluanid)	
3.1.1	Lot/Batch number	No lot or batch no. mentioned	
3.1.2	Specification	a) specific radioactivity was 33.7 µCi/mg [redacted] b) as given in section 2 of dossier [redacted]	
3.1.3	Purity	a) [redacted] radiochemical purity b) [redacted] purity	
3.1.4	Further relevant properties	-	
3.1.5	Method of analysis	The leachate was extracted twice with chloroform and twice with ethyl acetate. The chloroform phase and the ethyl acetate phase were worked up separately. The samples were analysed using Thin Layer Chromatography. Determination of ^{14}C Radioactivity: The volatile compounds were trapped in an oil-coated wool plug, extracted and measured by LSC. The $^{14}CO_2$ was passed into a cocktail and measured by LSC. The liquid samples were analyzed by LSC too. The soil samples were incinerated in an automatic combustion machine.	
3.2	**Reference substance**	Yes	**X**

Section A7.2.3.2 Aged residues soil leaching study

Annex Point IIIA XII 1.3

3.2.1	Method of analysis for reference substance	TLC	
3.3	**Soil types**	One soil was used (BBA Standard Soil 2.1), see table A7_2_3_2-1	**X**
3.4	**Testing procedure**		
3.4.1	Test system	The soil was sieved to a particle size ≤ 1 mm and dissolved dichlofluanid was applied via a soil sub sample. Incubation: From the treated sand soil 100 g dry weight samples were taken and placed in 8 incubation vessels. From the 8 samples, 2 were worked up immediately; further 2 vessels were subjected to leaching without ageing. The remaining 4 vessels were incubated under aerobic conditions for 30 or 90 days, respectively, with a connected trap to retain any volatile components. Leaching: Two columns were prepared with BBA standard soil 2.1 according to BBA bulletin 37 (height of soil column after compression 26 cm). After saturation with water, the soil sample incubated with dichlofluanid was put in a layer on top of the soil column and watered. The leachate was collected in 2 fractions of 200 ml. When the watering was finished the soil columns were deep-frozen and sliced into 3 pieces of equal length.	
3.4.2	Test solution and Test conditions	The radioactive labelled dichlofluanid was dissolved in ethyl acetate and mixed with unlabelled parent compound. The application rate (0.5 mg/column) was based on the maximum rate used in agricultural practice (2.5 kg/ha). In the soil samples that were immediately worked up, 1.05 µCi per column was recovered, corresponding to 0.525 mg a.i./column.	
		4 RESULTS	
4.1		See table A7_2_3_2-2	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Sand soil was incubated with dichlofluanid for 0, 30 and 90 days. Sub samples of the aged soil were then transferred to the top of soil columns containing untreated sand soil according to BBA Bulletin 37. The application rate was 0.525 mg dichlofluanid per column. The columns were then leached and the leachate was collected in 2 fractions of 200 ml.	
5.2	**Results and discussion**	The leachate contained up to 68% of the recovered radioactivity. Less than 1% unchanged parent compound was present in leachate; the major proportion was dimethylaminosulfanilide (DMSA). After 90 days of ageing the amount of radioactivity in the leachate was found to have declined to a level of 4%. DMSA was present only in small amounts (< 1%) in the leachate after 90 days of ageing. Over the same period period, 60% of applied dichlofluanid was degraded to CO_2.	X
5.3	**Conclusion**	Dichlofluanid can be classified as an immobile compound. The main metabolite dimethylaminosulfanilide (DMSA) is considered to be mobile.	
5.3.1	Reliability	2	

Section A7.2.3.2 Aged residues soil leaching study

Annex Point IIIA XII 1.3

5.3.2	Deficiencies	Batch numbers of test compound not given

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	09-11-04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	

Section A7.2.3.2 Aged residues soil leaching study

Annex Point IIIA XII 1.3

Remarks	[redacted]
	COMMENTS FROM ...
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	

Table A7_2_3_2-1: Classification and physico-chemical properties of the soil used

	BBA Standard Soil 2.1
Location	Speyer, Germany
Soil texture	sand
Sand [%]	87
Silt [%]	9
Clay [%]	4
Organic carbon [%]	0.69
pH (0.01 M $CaCl_2$)	7.0
Biomass at start of study [mg microbial C/kg dry weight soil]	24

Table A7_2_3_2-2: Distribution of dichlofluanid residues following soil column leaching. Figures are in % of radioactivity applied to column.

		Ageing time in days		
Radioactivity		0	30	90
Soil	upper third	9.5	22.0	34.5
	middle third	8.0	3.5	2.5
	lower third	17.0	5.5	1.0
Leachate	total	65.5	65.5	3.0
	Fraction I	< 0.1	1.5	< 1.0
	Fraction II, total	65.5	64.0	3.0
	Dichlofluanid in Fraction II	-	-	*< 1.0*
	Dimethylaminosulfanilide in Fraction II	*65.5*	*62.0*	-
	Unknown Metabolite M I in Fraction II	-	*< 1.0*	-
	Unknown Metabolite M II in Fraction II	-		*2.5*
	Aqueous phase after extraction	*< 1.0*	*2.0*	-
[$^{14}CO_2$]		-	3.0	59.0
Other volatile compounds		-	< 1.0	< 0.1
total recovered radioactivity [%]		100	100	100

Section A7.2.3.2 Soil leaching study

Annex Point IIIA XII 1.3

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1987, Leaching characteristics of Dichlofluanid (Euparen®) with various modes of application. [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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, Federal German Biological Agency for Agriculture and Forestry (BBA), Bulletin No. 37, 3rd edition (1984).	
2.2	**GLP**	GLP requirements of 40 CFR Part 160 do not apply to the study described in this document	
2.3	**Deviations**	No	
		3 MATERIALS AND METHODS	
3.1	**Test material**	a) [U^{14}C-phenyl]-dichlofluanid b) 50 WP [U^{14}C-phenyl]-dichlofluanid c) non-active standard substance (dichlofluanid)	
3.1.1	Lot/Batch number	Not given	
3.1.2	Specification	a) specific radioactivity was 1247 kBq/mg [redacted] b) specific radioactivity was 623 mBq/mg [redacted] c) as given in section 2 of dossier [redacted]	
3.1.3	Purity	a) [redacted] adiochemical purity b) [redacted] adiochemical purity c) [redacted] purity	
3.1.4	Further relevant properties	50 WP (Wettable Powder) is an agricultural formulation of dichlofluanid.	
3.1.5	Method of analysis	Fraction I (the first 200 ml of leachate) was not subjected to further analysis because the radioactivity level was too low. Fraction II (the second 200 ml) from variants 2B and 3A was extracted twice with chloroform. The chloroform phase was concentrated on a rotary evaporator, taken up in 1ml chloroform, and analysed using Thin Layer Chromatography. Determination of ^{14}C Radioactivity: The volatile compounds were	X

Section A7.2.3.2 Soil leaching study

Annex Point IIIA XII 1.3

		trapped in a oil-coated wool plug, extracted and measured by LSC. The $^{14}CO_2$ was passed into a cocktail and measured by LSC. The liquid samples were analysed by LSC too. The soil samples were incinerated in an automatic combustion machine.	
3.2	**Reference substance**	Yes, Dimethylaminosulphanilide (DMSA), the main metabolite was analysed in parallel as a reference compound.	**X**
3.2.1	Method of analysis for reference substance	TLC; the TLC findings were confirmed by mass-spectrometric analysis.	
3.3	**Soil types**	One soil was used (BBA Standard Soil 2.1), see table A7_2_3_2-1	**X**
3.4	**Testing procedure**		
3.4.1	Test system	The soil was sieved to a particle size ≤ 1 mm; three experimental variants were performed (see table A7_2_3_2-2). *Experimental variants:* In variant 1 the WP formulation was suspended in 1ml water and applied drop wise to the surface of the leach column. In variant 2 the WP formulation was worked into the soil via a soil sub sample. In variant 3 the unformulated active ingredient was worked into the soil via a soil sub sample. *Leaching:* Two columns were prepared with BBA standard soil 2.1 according to BBA bulletin 37 (height of soil column after compression 26 cm). The columns were watered with about 400 ml of water over a period of 48 h. The leachate was collected in 2 fractions of 200 ml. When the watering was finished the soil columns were deep-frozen. The columns from variants 1B, 2B and 3A were sliced into 3 pieces of equal length.	
3.4.2	Test solution and Test conditions	The WP formulation was suspended in water (variants 1 and 2) In variant 3 the radioactive labelled dichlofluanid was dissolved in ethyl acetate and mixed with unlabelled parent compound. The application rate (0.5 mg/column) was based on the maximum rate used in agricultural practice (2.5 kg/ha). This application rate is equivalent to 0.5 mg active ingredient per column.	
		4 RESULTS	
4.1		See table A7_2_3_2-3	**X**
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	Dichlofluanid WP 50 formulation and unformulated active ingredient were dropped on the surface of soil columns or sub samples of treated soil were transferred to the top of soil columns containing untreated sand soil according to BBA Bulletin 37. The application rate was 0.5 mg dichlofluanid per column. The columns were then leached for 48 h with 400 ml water and the leachate was collected in 2 fractions of 200 ml.	

Section A7.2.3.2 Soil leaching study

Annex Point IIIA XII 1.3

5.2	**Results and discussion**	After drop wise application of the suspended formulation on the surface of the soil in the leach-column, the leachate contained fewer radioactivities in the 2-day test than after incorporation of the formulation into the upper part of the soil column. The percentage of radioactivity in the leachate was less for the trial with incorporated formulation than for the trial with non-formulated incorporated compound. The leachate contained no unchanged parent compound (< 0.1 %); the major proportion was dimethylaminosulfanilide (DMSA). DMSA occurred in the leachate in amounts ranging from 1% (formulation, not incorporated) to 30% (active ingredient, incorporated) of the originally applied radioactivity, depending on the method of application used.	X
5.3	**Conclusion**	Dichlofluanid can be classified as an immobile compound.	
5.3.1	Reliability	2	
5.3.2	Deficiencies	Batch numbers of test compound not given	

Section A7.2.3.2 Soil leaching study

Annex Point IIIA XII 1.3

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	11/10/2004
Materials and Methods	[redacted]

Section A7.2.3.2 Soil leaching study

Annex Point IIIA XII 1.3

Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	

	COMMENTS FROM ...
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	

Table A7_2_3_2-1: Classification and physico-chemical properties of the soil used

	BBA Standard Soil 2.1
Location	Speyer, Germany
Soil texture	sand
Suspendable fraction [%]	10.7
Organic carbon [%]	0.69
pH (0.01 M $CaCl_2$)	7.0
Max. water capacity [%]	18.2
Biomass at start of study [mg microbial C/kg dry weight soil]	106

Table A7_2_3_2-2: Experimental variants of the leaching experiment and radioactivity in the leachates

Experi-mental variant	Experimental conditions				Result
	Method of application	mg a.i/ soil column	mg product/ soil column	Quantity of radioactivity applied [kBq]	Radioactivity in leachate [% of applied radioactivity]
1 A	50 WP in 1 ml water applied drop wise to soil	0.49	0.98	610.6	0.4
1 B		0.50	1.00	623.3	1.3
2 A	50 WP worked into the soil via a sub sample	0.49	0.97	605.2	7.6
2 B		0.49	0.98	610.2	11.5
3 A	a.i. worked into the soil via a sub sample	0.49	-	40.9	32.4
3 B		0.49	-	40.9	28.1

Table A7_2_3_2-3: Distribution of dichlofluanid residues following soil column leaching. Figures are in % of radioactivity applied to column (= 100 %)

		Experimental variant					
Radioactivity		**1 A**	**1 B**	**2 A**	**2 B**	**3 A**	**3 B**
I. Soil	upper third	--	78.9	--	52.2	16.3	--
	middle third	--	10.6	--	14.5	23.3	--
	lower third	--	5.1	--	13.7	28.2	--
II. Leachate	total	0.4	1.3	7.6	11.5	32.4	28.1
	Fraction I	--	0.01	< 0.1	0.2	0.1	< 0.1
	Fraction II, total	--	1.3	7.6	11.3	32.3	28.1
	Dichlofluanid in Fraction II	--	--	--	*< 0.1*	*< 0.1*	--
	Dimethylaminosulfanilide in Fraction II	--	--	--	*10.0*	*32.0*	--
	Unidentified Radioactivity	--	--	--	*< 0.1*	*0.1*	--
	Aqueous phase after extraction	--	--	--	*1.3*	*0.3*	--
Sum I. + II. [%]		--	95.9	--	91.9	100.2	--
Sum I. + II. [kBq]		--	597.7	--	560.8	41.0	--
Total applied radioactivity [kBq]		610.6	623.3	605.3	610.2	40.9	40.9

--: This experimental variant was not investigated

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1986, Acute Flow – Through Toxicity of Preventol A4-S to Bluegill Sunfish (*Lepomis macrochirus*) [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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 U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (1975)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, after comparison with OECD guideline No. 203: Observation for mortality was not made in blank control	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier	
3.1.1	Lot/Batch number	Lot number: N 112/1835 K	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see table A7_4_1_1-1	
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_1_1-2	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

3.4.2	Test organisms	see table A7_4_1_1-3	X
3.4.3	Test system	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 hours	
3.4.6	Test parameter	Mortality and sublethal responses	
3.4.7	Sampling	Observations for mortality and sublethal responses were made once every 24 hours (each test level and acetone solvent control). Dead individuals were removed at each observation period. Temperature, dissolved oxygen and pH were measured in the solvent control, the low and the highest test concentration which contained surviving fish at 0, 48 and 96 hours.	X
3.4.8	Monitoring of TS concentration	Yes, at 0 and 96 hours	X
3.4.9	Statistics	Statistical analysis of results for 24, 48, 72 and 96 – hour LC_{50} values and their corresponding 95% confidence limits was obtained by employing a LC_{50} computerized program using the binomial, the moving average and the probit method.	
		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: 1.0, 0.5, 0.25, 0.125 and 0.06 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0.50, 0.25, 0.10, 0.05 and 0.024 mg/l	X
4.2.3	Effect data (Mortality)	see table A7_4_1_1-6 and table A7_4_1_1-7	
4.2.4	Concentration / response curve	No graph is given in the report	
4.2.5	Other effects	Sublethal/behavioural responses (e.g. loss of equilibrium, bottom orientation and rapid respiration) were observed in the 0.10 and 0.05	

		mg/l test levels.	
4.3	**Results of controls**		X
4.3.1	Number/ percentage of animals showing adverse effects	No mortality occurred in the solvent control	
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**	A 96 - hour flow - through study was conducted in accordance with the guideline U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (1975) in order to estimate the acute toxicity of dichlofluanid to bluegill sunfish (*Lepomis macrochirus*). Comparison with OECD guideline No. 203 shows no relevant deviations except that observation for mortality was not made in blank control.	
5.2	**Results and discussion**	A 96 – hour LC_{50} value was calculated to be 0.030 mg/l with 95% confidence limits ranging from 0.024 to 0.050 mg/l. The result is based on the measured test concentrations of dichlofluanid. A 96 – hour no effect concentration of dichlofluanid was determined to be < 0.024 mg/l, because all test concentrations elicited total or partial mortality. No mortality occurred in the solvent control. The determination of the test substance concentrations in the test system showed low analytical results.	
5.2.1	96h-LC_0	< 0.024 mg/l	X
5.2.2	96h-LC_{50}	0.030 mg/l	
5.2.3	96h-LC_{100}	0.05 mg/l	
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_1-8. The measured concentrations of test substance are not ≥ 80% of nominal concentrations during the test. The differences between the nominal and measured concentrations were likely due to the fact that dichlofluanid is very rapidly hydrolysed in aqueous solutions. A concentration/response curve is not available, but a dose – response relationship can be seen from the experiment.	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

5.3.1	Other Conclusions	-
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes, observation for mortality was not made in blank control, no graph is given in the report

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/01/05 [redacted]
Materials and Methods	[redacted]
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Lepomis macrochirus*

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	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes A diluter stock solution (17.500 mg/l) was prepared by dissolving 1.750 g of dichlofluanid in 100 ml of acetone.
Concentration of vehicle	Concentration in solvent control: 0.05 ml/l
Vehicle control performed	Yes Observation for mortality and sublethal responses was performed in solvent control
Other procedures	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	[redacted]
Alkalinity	325 – 375 mg/l
Hardness	225 – 275 mg/l
pH	7.8 – 8.3
Oxygen content	9.2 – 10.1 mg/l (after aeration)
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Bluegill Sunfish (*Lepomis macrochirus*)
Source	[redacted]
Wild caught	No
Age/size	Bluegill sunfish used as control group: mean weight of 1.5 (± 0.4) g and a mean standard length of 46 (± 4.5) mm.
Kind of food	The fish [redacted] were fed newly hatched brine shrimp or a commercially available trout food
Amount of food	-
Feeding frequency	Daily
Pretreatment	72 hours before initiation of test, fish were placed in the temperature acclimation unit and held without food during this time.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	1 litre of test solution or control water was delivered to the test vessels at an average rate of 15 times per hour over the course of the study. This flow rate was sufficient to replace the 15 litre volume within the test chambers 24 times per day.
Volume of test vessels	15 l
Volume/animal	750 ml
Number of animals/vessel	20
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	22 – 23 °C
Dissolved oxygen	8.8 – 9.1 mg/l
pH	7.9 – 8.2
Adjustment of pH	No
Aeration of dilution water	Yes (pretreatment)
Intensity of irradiation	-
Photoperiod	Laboratory environment was maintained on a 16-hour daylight photoperiod

Table A7_4_1_1-6: Mortality data

Test Substance Measured Concentration [mg/l][1]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Solvent control	0	0	0	0	0	0	0	0
0.024	4	4	4	4	20	20	20	20
0.05	12	19	19	20	60	95	95	100
0.10	19	20	20	20	95	100	100	100
0.25	20	20	20	20	100	100	100	100
0.50	20	20	20	20	100	100	100	100
Temperature [°C]	22 - 23							
pH	7.9 – 8.2							
Oxygen [mg/l]	8.8 – 9.1							

[1] Test substance concentrations are mean measured concentrations

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	< 0.024	-	< 0.024	-
LC_{50}	0.031	0.026 – 0.037	0.030	0.024 – 0.05
LC_{100}	0.10	-	0.05	-

[1] Effect data are based on measured concentrations

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

Criteria for poorly soluble test substances	X	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Salmo gairdneri*

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1986, Acute Flow – Through Toxicity of Preventol A4-S to Rainbow Trout (*Salmo gairdneri*) [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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	
		U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (1975)	
2.2	**GLP**	Yes	
2.3	**Deviations**	Yes, after comparison with OECD guideline No. 203:	
		Observation for mortality was not made in blank control	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier	
3.1.1	Lot/Batch number	Lot number: N 112/1835 K	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	see table A7_4_1_1-1	
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_1_1-2	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Salmo gairdneri*

3.4.2	Test organisms	see table A7_4_1_1-3	X
3.4.3	Test system	see table A7_4_1_1-4	
3.4.4	Test conditions	see table A7_4_1_1-5	X
3.4.5	Duration of the test	96 hours	
3.4.6	Test parameter	Mortality and sublethal responses	
3.4.7	Sampling	Observations for mortality and sublethal responses were made once every 24 hours (each test level and acetone solvent control). Dead individuals were removed at each observation period. Temperature, dissolved oxygen and pH were measured in the solvent control, the low and the highest test concentration containing surviving fish at 0, 48 and 96 hours.	X
3.4.8	Monitoring of TS concentration	Yes, at 0 and 96 hours	X
3.4.9	Statistics	Statistical analysis of results for 24, 48, 72 and 96 – hour LC_{50} values and their corresponding 95% confidence limits was obtained by employing a LC_{50} computerized program using the binomial test.	
		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: 0.1, 0.05, 0.025, 0.012 and 0.006 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0.033, 0.016, 0.0066, < 0.0026 and < 0.0026 mg/l	X
4.2.3	Effect data (Mortality)	see table A7_4_1_1-6 and table A7_4_1_1-7	
4.2.4	Concentration / response curve	The mortality increases from 0% to 100% between doses of 0.0066 mg/l (0% mortality) and 0.016mg/l (100% mortality). The presentation of a concentration/response curve is therefore not useful.	
4.2.5	Other effects	Sublethal/behavioural responses (e.g. surfacing, bottom orientation and loss of equilibrium) were noted among the fish in the 0.016 and 0.0066	

		mg/l test levels.	
4.3	**Results of controls**		X
4.3.1	Number/ percentage of animals showing adverse effects	No mortality occurred in the solvent control	
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**	A 96 - hour flow - through study was conducted in accordance with the guideline U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (1975) in order to estimate the acute toxicity of dichlofluanid to rainbow trout (*Salmo gairdneri*). Comparison with OECD guideline No. 203 shows no relevant deviations except that observation for mortality was not made in blank control.	
5.2	**Results and discussion**	A 96 – hour LC_{50} value was calculated to be 0.010 mg/l with 95% confidence limits ranging from 0.0066 to 0.016 mg/l. The result is based on the measured test concentrations of dichlofluanid. A 96 – hour no effect concentration of dichlofluanid was determined to be < 0.0026 mg/l, based on a lack of sublethal responses. No mortality occurred in the solvent control. The determination of the test substance concentrations in the test system showed low analytical results.	
5.2.1	96h-LC_0	< 0.0026 mg/l	
5.2.2	96h-LC_{50}	0.010 mg/l	X
5.2.3	96h-LC_{100}	0.016 mg/l	
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_1-8. The measured concentrations of test substance are not ≥ 80% of nominal concentrations during the test. The differences between the nominal and measured concentrations were likely due to the fact that dichlofluanid is very rapidly hydrolysed in aqueous solutions. A dose – response curve is not given, but it can be seen from the results that this curve must be very steep since the mortality increases from 0% to 100% between doses of 0.0066 mg/l (0% mortality) and 0.016 mg/l (100% mortality).	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Salmo gairdneri*

5.3.1	Other Conclusions	-
5.3.2	Reliability	2
5.3.3	Deficiencies	Yes, observation for mortality was not made in blank control

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/12/04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	

Section A7.4.1.1 Acute toxicity to fish

Annex Point IIA VII.7.1 *Salmo gairdneri*

Acceptability	[redacted]
Remarks	
	COMMENTS FROM ...
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	

Table A7_4_1_1-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	No
Vehicle	Yes A diluter stock solution (2000 mg/l) was prepared by dissolving 0.200 g of dichlofluanid in acetone.
Concentration of vehicle	Concentration in solvent control: 0.05 ml/l
Vehicle control performed	Yes Observation for mortality and sublethal responses was performed in solvent control
Other procedures	-

Table A7_4_1_1-2: Dilution water

Criteria	Details
Source	[redacted]
Alkalinity	325 – 375 mg/l
Hardness	225 – 275 mg/l
pH	7.8 – 8.3
Oxygen content	9.2 – 10.1 mg/l (after aeration)
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_1-3: Test organisms

Criteria	Details
Species/strain	Rainbow trout (*Salmo gairdneri*)
Source	[redacted]
Wild caught	No
Age/size	Rainbow trout used as control group: mean weight of 0.35 (± 0.071) g and a mean standard length of 36 (± 2.3) mm.
Kind of food	The fish were reared [redacted] fed newly hatched brine shrimp or a commercially available trout food
Amount of food	-
Feeding frequency	Daily
Pretreatment	96 hours before initiation of test, trout were placed in the temperature acclimation unit and held without food during this time.
Feeding of animals during test	No

Table A7_4_1_1-4: Test system

Criteria	Details
Test type	Flow-through
Renewal of test solution	1 litre of test solution or control water was delivered to the test vessels at an average rate of 7 times per hour over the course of the study. This flow rate was sufficient to replace the 15 litre volume within the test chambers 11 times per day.
Volume of test vessels	15 l
Volume/animal	750 ml
Number of animals/vessel	20
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	12 – 13 °C
Dissolved oxygen	9.1 – 9.3 mg/l
pH	8.0 – 8.2
Adjustment of pH	No
Aeration of dilution water	Yes (pretreatment)
Intensity of irradiation	-
Photoperiod	Laboratory environment was maintained on a 16-hour daylight photoperiod

Table A7_4_1_1-6: Mortality data

Test Substance Measured Concentration [mg/l][1]	Mortality							
	Number				Percentage			
	24 h	48 h	72 h	96 h	24 h	48 h	72 h	96 h
Solvent control	0	0	0	0	0	0	0	0
< 0.0026	0	0	0	0	0	0	0	0
< 0.0026	0	0	1	1	0	0	5	5
0.0066	0	0	0	0	0	0	0	0
0.016	12	20	20	20	60	100	100	100
0.033	20	20	20	20	100	100	100	100
Temperature [°C]	12 - 13							
pH	8.0 – 8.2							
Oxygen [mg/l]	9.1 – 9.3							

[1] Test substance concentrations are mean measured concentrations

Table A7_4_1_1-7: Effect data

	48 h [mg/l][1]	95 % c.l.	96 h [mg/l][1]	95 % c.l.
LC_0	0.0066	-	< 0.0026	-
LC_{50}	0.010	0.0066 – 0.016	0.010	0.0066 – 0.016
LC_{100}	0.016	-	0.016	-

[1] Effect data are based on measured concentrations

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

Criteria for poorly soluble test substances	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] 1986, Acute flow-through toxicity of Preventol A4-S to *Daphnia magna* [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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, U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (April 1975)	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 202	
		3 MATERIALS AND METHODS	
3.1	**Test material**	As given in section 2 of dossier	
3.1.1	Lot/Batch number	Lot number: N 112/1835 K	
3.1.2	Specification	As given in section 2 of dossier	
3.1.3	Purity	[redacted]	X
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	-	
3.1.6	Method of analysis	HPLC	
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	See table A7_4_1_2-1	
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_1_2-2	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

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	
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Mortality and behavioural observation	
3.4.7	Sampling	Mortality and behavioural observation was performed at 24 and 48 hours; pH and dissolved oxygen concentration of test samples (control, low, middle and high concentrations of test substance) were controlled at 0 and 48 hours	X
3.4.8	Monitoring of TS concentration	Yes, at 0 and 48 hours	X
3.4.9	Statistics	Statistical analysis was obtained by employing a computerized program. The LC_{50} values were calculated using the moving average method.	
		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: 0.12, 0.19, 0.40, 0.69, 1.6 mg/l	
4.2.2	Actual concentrations of test substance	Measured concentrations (mean values): 0.071, 0.099, 0.24, 0.35, 1.0 mg/l	X
4.2.3	Effect data (Immobilisation)	see table A7_4_1_2-6 and table A7_4_1_2-7	X
4.2.4	Concentration / response curve	No graph is given in the report	X
4.2.5	Other effects	Abnormal/behavioural responses (e.g. surfacing, quiescence and bottom orientation) were noted among the daphnids in the 0.099, 0.24, 0.35 and 1.0 mg/l test substance concentrations.	
4.3	**Results of controls**	No mortality occurred in the controls	
4.4	**Test with**	Not performed	

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

	reference substance	
4.4.1	Concentrations	-
4.4.2	Results	-

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Acute toxicity test to *Daphnia magna* was performed in accordance with guideline U.S.-EPA, Ecological Research Series EPA-660/3-75-009, (April 1975). The test, performed in a flow-through system, prolonged to 48 hours. Comparison with OECD guideline No. 202 shows no relevant deviations.
5.2	**Results and discussion**	A LC_{50} value of 0.57 mg/l at 24 hours was shown in the test. The result is based on measured test concentrations used for statistical analysis. The 48-hour no-effect concentration was 0.071 mg/l, based on the lack of mortality and abnormal effects. No mortality occurred in the controls. The determination of the test substance concentrations in the test system showed low analytical results.
5.2.1	LC_0	0.099 mg/l after 24 h and 0.071 after 48 h
5.2.2	LC_{50}	0.57mg/l after 24 h, and 0.42 mg/l after 48 h
5.2.3	LC_{100}	> 1.0 mg/l after 24 h, and 1.0 mg/l after 48 h
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-8. The measured concentrations of test substance are not ≥ 80% of nominal concentrations during the test. The differences between the nominal and measured concentrations were likely due to the fact that dichlofluanid is very rapidly hydrolysed in aqueous solutions. A concentration/response curve is not available but a dose – response relationship can be seen from the experiment.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes It must be noted that the LC_{50} value was calculated instead of the EC_{50} value. Therefore the EC_{50} value based on immobilisation is lower than 0.42 mg/l after 48 hours. Information is incomplete about test organism, No concentration/response curve available

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	28/01/05
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
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*

Section A7.4.1.2 Acute toxicity to invertebrates

Annex Point IIA VII.7.2 *Daphnia magna*

Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A7_4_1_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes special device (mixing box)
Vehicle	Yes dimethylformamide was used in the preparation of all working stock solutions
Concentration of vehicle	Volume for preparation of stock solution: 100 ml
Vehicle control performed	Yes mortality and behavioural observation was performed in solvent control
Other procedures	-

Table A7_4_1_2-2: Dilution water

Criteria	Details
Source	[redacted] well water
Alkalinity ($CaCO_3$)	325-375 mg/l
Hardness ($CaCO_3$)	225-275 mg/l
pH	7.8 – 8.3
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	9.2- 10.1 mg/l
Conductance	700 µmhos/cm
Holding water different from dilution water	No

Table A7_4_1_2-3: Test organisms

Criteria	Details
Strain	*Daphnia magna*
Source	[redacted]
Age (at start of the study)	< 24 – hours old
Breeding method	-
Kind of food	Suspension of algae (*Selenastrum capricornutum*) supplemented with a yeast suspension
Amount of food	-
Feeding frequency	-
Pretreatment	-
Feeding of animals during test	During the holding period daphnids were fed with the above named kind of food

Table A7_4_1_2-4: Test system

Criteria	Details
Renewal of test solution	Flow-through system: aerated [redacted] well water was delivered to each test chamber at a rate of 125 ml/chamber /10 minutes, an amount which was sufficient to replace the 1-liter test volume approximately 19 times in a 24-hour period.
Volume of test vessels	1 l
Volume/animal	100 ml
Number of animals/vessel	10
Number of vessels/ concentration	4 (4 replicate test chambers, i.e. 40 daphnids were used per concentrations)
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 – 21 °C
Dissolved oxygen	8.3 – 8.7 mg/l
pH	8.2 – 8.3
Adjustment of pH	No
Aeration of dilution water	Yes pretreatment
Quality/Intensity of irradiation	50 – 70 footcandles
Photoperiod	16 – hour daylight photoperiod, with 30 minutes dawn and dusk transition periods

Table A7_4_1_2-6: Mortality data

Test Substance Concentration (effective) [1] [mg/l]	Mortality of *Daphnia*						
	Number		Percentage		Oxygen [mg/l]	pH	Temperature [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	8.3	8.2	20
Solvent control	0	0	0	0			
0.071	0	0	0	0	8.5	8.3	20
0.099	0	1	0	2.5			
0.24	1	2	2.5	5	8.5	8.3	20
0.35	3	16	7.5	40			
1.0	38	40	95	100	8.7	8.3	20

[1] Test substance concentrations are mean measured concentrations

Table A7_4_1_2-7: Effect data *

	LC_{50} [1]	95 % c.l.	LC_0 [1]	LC_{100} [1]
24 h [mg/l]	0.57	0.51 – 0.67	0.099	> 1.0
48 h [mg/l]	0.42	0.37 – 0.47	0.071	1.0

[1] Effect data are based on measured concentrations

* The LC_{50} value was calculated instead of the EC_{50} value

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 of

Annex Point IIA VII.7.2 **DIMETHYLAMINOSULFANILID (DMSA)**
Daphnia magna

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] 1997, Dimethylaminosulfanilid (DMSA) Acute Daphnia Toxicity [redacted]	
1.2	**Data protection**	Yes	
1.2.1	Data owner	[redacted]	
1.2.2	Companies with letter of access	[redacted]	
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, Study was performed in accordance with Council Directive 92/69/EEC, Part C.2. This test method is in most parts identical with OECD guideline No. 202	
2.2	**GLP**	Yes	
2.3	**Deviations**	No, the study is comparable to OECD guideline No. 202	
		3 MATERIALS AND METHODS	
3.1	**Test material**	Dimethylaminosulfanilid (DMSA)	
3.1.1	Lot/Batch number	Article number: 00436151	
3.1.2	Specification		
3.1.3	Purity	[redacted]	
3.1.4	Composition of Product	-	
3.1.5	Further relevant properties	water solubility = 2 g/l at 20 °C	
3.1.6	Method of analysis	No data	X
3.2	**Preparation of TS solution for poorly soluble or volatile test substances**	The test substance was added directly to the test water without the use of solvents and distributed by ultrasonic bath and magnetic stirrer.	
3.3	**Reference substance**	No	
3.3.1	Method of analysis for reference substance	-	
3.4	**Testing procedure**		

Section A7.4.1.2 Acute toxicity to invertebrates of

Annex Point IIA VII.7.2 **DIMETHYLAMINOSULFANILID (DMSA)**
Daphnia magna

3.4.1	Dilution water	see table A7_4_1_2-1	X
3.4.2	Test organisms	see table A7_4_1_2-2	X
3.4.3	Test system	see table A7_4_1_2-3	X
3.4.4	Test conditions	see table A7_4_1_2-4	X
3.4.5	Duration of the test	48 hours	
3.4.6	Test parameter	Immobilisation	
3.4.7	Sampling	Immobilisation of Daphnia is recorded at the start, after 24 hours and at the end of the study. Water temperature, pH and oxygen values are measured at the end of the study. The concentrations of the C-containing components of the test medium were confirmed by TOC determination at the start and end of the study.	X
3.4.8	Monitoring of TS concentration	Yes, at the start and end of the test	
3.4.9	Statistics	The EC_0 was determined directly from the study	
		4 RESULTS	
4.1	**Limit Test**	Performed	
4.1.1	Concentration	100 mg/l	
4.1.2	Number/ percentage of animals showing adverse effects	No immobilisation of daphnids occurred in the test level.	
4.1.3	Nature of adverse effects	-	
4.2	**Results test substance**		
4.2.1	Initial concentrations of test substance	Nominal concentration: 100 mg/l (limit test)	
4.2.2	Actual concentrations of test substance	Measured concentrations: 94.5 mg/l at 0 hours, 96.6 mg/l at 48 hours, Average: 95.6 mg/l	
4.2.3	Effect data (Immobilisation)	see table A7_4_1_2-5 and table A7_4_1_2-6	
4.2.4	Concentration / response curve	No immobilisation occurred during the test. Therefore no concentration / response curve is given in the report.	
4.2.5	Other effects	-	

Section A7.4.1.2 Acute toxicity to invertebrates of

Annex Point IIA VII.7.2 **DIMETHYLAMINOSULFANILID (DMSA)**
Daphnia magna

4.3	**Results of controls**	No immobilisation occurred in 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**	To assess the acute toxic effects (immobilisation) of dimethylamino-sulfanilid (DMSA) on *Daphnia magna*, a 48-hour limit test under static conditions was performed. The study was conducted in accordance to the Council Directive 92/69/EEC, C.2, which is in most parts identical with the OECD guideline No. 202. Comparison with OECD guideline No. 202 shows no relevant deviations.
5.2	**Results and discussion**	The EC_0 of the test substance dimethylaminosulfanilid (DMSA) after 48 hours for the species *Daphnia magna* is $\geq$ 95.6 mg/l. No immobilisation occurred in the control and the 100 mg/l test level. The test substance was sufficiently stable under the test conditions. The analytical data show that the test concentration was over 80% of the theoretical value of 100 mg/l throughout the duration of the test.
5.2.1	EC_0	$\geq$ 95.6 mg/l after 48 hours
5.2.2	EC_{50}	-
5.2.3	EC_{100}	-
5.3	**Conclusion**	The validity criteria are summarised in table A7_4_1_2-7. The test fulfils the validity criteria of the OECD guideline No. 202.
5.3.1	Reliability	2
5.3.2	Deficiencies	Yes Information incomplete about dilution water, test organism, test system and test conditions. No method of analysis mentioned used for determination of the test substance concentration in the test vessel.

Section A7.4.1.2 Acute toxicity to invertebrates of

Annex Point IIA VII.7.2 **DIMETHYLAMINOSULFANILID (DMSA)**
Daphnia magna

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13/12/04
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	
Reliability	
Acceptability	
Remarks	
	COMMENTS FROM ...
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	

Table A7_4_1_2-1: Dilution water

Criteria	Details
Source	M4-medium according to BGA (1992)
Alkalinity ($CaCO_3$)	-
Hardness ($CaCO_3$)	274.9 mg/l $CaCO_3$ = 15.4 d°H
pH	-
Ca / Mg ratio	-
Na / K ratio	-
Oxygen content	-
Conductance	-
Holding water different from dilution water	No

Table A7_4_1_2-2: Test organisms

Criteria	Details
Strain	Daphnia magna STRAUS, parthenogenetic females
Source	[redacted]
Age (at start of the study)	0 – 24 hours
Breeding method	Keeping of Daphnia: M4-medium according to BGA (1992)
Kind of food	-
Amount of food	-
Feeding frequency	-
Pretreatment	-
Feeding of animals during test	No data