Committee for Risk Assessment
RAC

Annex 1
Background document
to the Opinion proposing harmonised classification and labelling at EU level of

cyflumetofen (ISO); 2-methoxyethyl (RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate

EC Number: -
CAS Number: 400882-07-7

CLH-O-0000001412-86-183/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

5 December 2017

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

Substance Name: Cyflumetofen

EC Number: **not allocated**

CAS Number: **400882-07-7**

Index Number: **none**

Contact details for dossier submitter:

Bureau REACH

National Institute for Public Health and the Environment (RIVM)

The Netherlands

bureau-reach@rivm.nl

Version number: 1.2   **Date: December 2016**

CONTENTS

Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1 Substance identity

Substance name:	cyflumetofen
EC number:	Not allocated
CAS number:	400882-07-7
Annex VI Index number:	-
Degree of purity:	975 g/kg (racemic; minimum purity)
Impurities:	No relevant impurities

1.2 Harmonised classification and labelling proposal

Table 2 The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	None
Current proposal for consideration by RAC	Skin Sens. 1A; H317 Carc. 2; H351
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Skin Sens. 1A; H317 Carc. 2; H351

ᵃ no harmonized classification according to Directive 67/548/EEC (Dangerous Substances Directive; DSD) required

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3 Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1]	Reason for no classification [2]
2.1.	Explosives	none		none	conclusive but not sufficient for classification
2.2.	Flammable gases	none		none	conclusive but not sufficient for classification
2.3.	Flammable aerosols	none		none	conclusive but not sufficient for classification
2.4.	Oxidising gases	none		none	conclusive but not sufficient for classification
2.5.	Gases under pressure	none		none	conclusive but not sufficient for classification
2.6.	Flammable liquids	none		none	conclusive but not sufficient for classification
2.7.	Flammable solids	none		none	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none		none	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none		none	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none		none	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none		none	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none		none	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none		none	conclusive but not sufficient for classification
2.14.	Oxidising solids	none		none	conclusive but not sufficient for

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification[1]	Reason for no classification [2]
					classification
2.15.	Organic peroxides	none		none	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none		none	data lacking
3.1.	Acute toxicity – oral	none		none	conclusive but not sufficient for classification
	Acute toxicity - dermal	none		none	conclusive but not sufficient for classification
	Acute toxicity - inhalation	none		none	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	none		none	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	none		none	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	none		none	data lacking
3.4.	Skin sensitisation	Skin Sens. 1A; H317			
3.5.	Germ cell mutagenicity	none		none	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc. 2; H351			
3.7.	Reproductive toxicity	none		none	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	none		none	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	none		none	conclusive but not sufficient for classification
3.10.	Aspiration hazard	none		none	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	none		none	conclusive but not sufficient for classification
5.1.	Hazardous to the ozone layer	none		none	conclusive but not sufficient for classification

[1] Including specific concentration limits (SCLs) and M-factors

[2] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Warning

Symbol: GHS07, GHS08
Hazard statements: Skin Sens. 1A, H317: May cause an allergic skin reaction
                              Carc. 2, H351: Suspected of causing cancer
Precautionary statements: not included in Annex VI of CLP

Proposed notes assigned to an entry:

None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Cyflumetofen is a new active substance for plant protection products and currently has no harmonised classification.

2.2 Short summary of the scientific justification for the CLH proposal

Classification for physico - chemical hazards

Cyflumetofen does not fulfil any of the criteria for classification for physical hazards deemed relevant for a solid substance. No experimental data are available for the corrosion to metals endpoint as no relevant test design presently exists for solid substances.

Classification for human health hazards

- Based on the results of a maximization study in guinea pigs (sensitizing effect in 100% of the test animals after an intradermal induction dose of 1%), it is concluded that cyflumetofen should be classified for skin sensitization as <u>Skin Sens. 1A (H317: May cause an allergic skin reaction)</u>.

- With respect to the potential carcinogenic effect of cyflumetofen in humans, the following justification is presented:
    - Classification in category 1A is not considered for cyflumetofen as there are no human data regarding carcinogenicity.
    - Classification in category 1B is not considered for cyflumetofen. The available data cannot be considered as sufficient evidence which is defined as "an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more species" (CLP Regulation, section 3.6.2.2.3.b). These criteria are not met.
    - An increased incidence in thyroid C-cell carcinoma/adenoma was observed in Fischer F344 rats, which was above concurrent and historical controls. This effect is considered treatment-related and suggests a carcinogenic effect of cyflumetofen. Given that the evidence of carcinogenicity is restricted to a single experiment and the potential relevance for humans can be questioned, the irrelevance is not demonstrated for the cyflumetofen-induced thyroid tumours in rat, it can therefore be concluded there is limited evidence for carcinogenic effects of cyflumetofen. Classification for carcinogenicity as <u>Carc 2 (H351: Suspected of causing cancer)</u> is therefore proposed.

Classification for environmental hazards

Cyflumetofen needs not be classified for environmental hazards.

2.3   Current harmonised classification and labelling

2.3.1 Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

None

2.3.2 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

None

2.4   Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

The self-classification according to the inventory of notified classification and labelling on October 2015 was:

Classification			Labelling		Specific Concentration limits, M-Factors	Notes	Number of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code(s)			
Skin Sens. 1	H317	H317		GHS07 GHS09 Wng			23
Acute Tox. 4	H332	H332					
Aquatic Acute 1	H400	H400					
Skin Sens. 1	H317	H317		GHS07 Wng			2

2.4.2 Current self-classification and labelling based on DSD criteria

The inventory of notified classification and labelling does not contain the self-classification according to the DSD criteria. There is no registration (October 2015).

RAC general comment

Cyflumetofen is a bridged diphenyl acaricide (or benzoylacetonitrile acaricide), with a mode of action that is selective towards spider mites. It interferes with energy production (inhibition of complex II in mitochondria) on contact with *Tetranychyus urticae* (red spider mite). It has been approved in accordance with Regulation (EC) No 1107/2009 (Commission Implementing Regulation (EU) No 22/2013 of 15 January 2013). The representative uses evaluated comprise both indoor and outdoor spray application to ornamental crops, nursery trees, perennial ornamentals and to public greens. Cyflumetofen is a racemic mixture, but the specific metabolism or degradation of the individual enantiomers in experimental animals was not investigated.
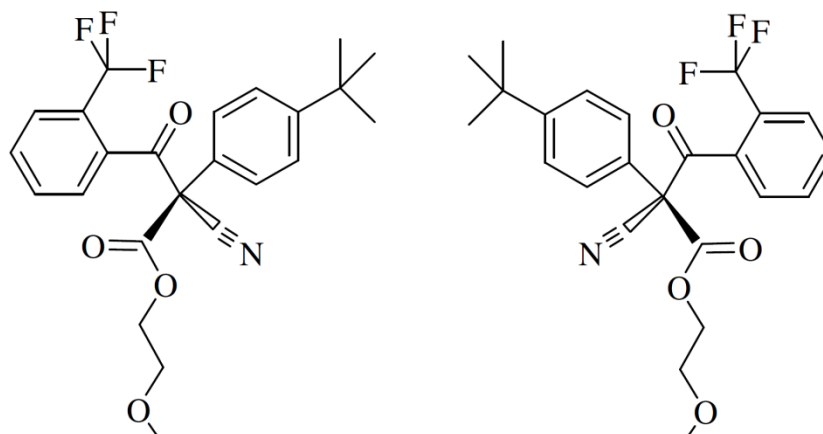
S-cyflumetofen   *R*-cyflumetofen

Cyflumetofen has no current entry in Annex VI of the CLP regulation and all hazard classes are open for assessment.

In rat, following oral administration, absorption of cyflumetofen was 61-67% or 15-26% after single low dose (3 mg/kg bw) or high dose (250 mg/kg bw), respectively, based on radiolabel recovered from urine, tissues and residual carcasses, measured 72 hours after administration. Radiolabeled cyflumetofen was mainly distributed to liver and kidneys. After 4 daily doses equilibrium was achieved, and no bioaccumulation occurred in any of the tissues and organs investigated. Cyflumetofen was extensively metabolized; displaying glucuronic acid conjugation, glutathione conjugation, mercapturic acid conjugation and thiolactic acid conjugation. Excretion was rapid with 80% or more of the administered radiolabel within 72 hours, irrespective of sex or dosing regimen. Most radiolabel was excreted via urine at low dose, whereas after high dose administration most radiolabel was excreted in faeces.

There was a significant increase in the amount of parent compound excreted via faeces after repeated dosing compared to single dosing. This suggests saturation of absorption following a repeated dosing regimen.

3   JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Cyflumetofen is an active substance for plant protection products and was approved under Regulation (EC) No 1107/2009 via Commission Implementing Regulation (EU) No 22/2013 and subsequently amending the Annex to Commission Implementing Regulation (EU) No 540/2011. Cyflumetofen is therefore subject to harmonised classification and labelling according to article 36.2 of CLP.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1   IDENTITY OF THE SUBSTANCE

1.1   Name and other identifiers of the substance

 Table 4 Substance identity

EC number:	Not allocated
EC name:	Not allocated
CAS number (EC inventory):	-
CAS number:	400882-07-7
CAS name:	Benzenepropanoic acid, α-cyano-α-[4-(1,1-dimethylethyl)phenyl]-β-oxo-2-(trifluoromethyl)-, 2-methoxyethyl ester
IUPAC name:	2-methoxyethyl (*RS*)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate
ISO name:	cyflumetofen
CLP Annex VI Index number:	-
Molecular formula:	$C_{24}H_{24}F_3NO_4$
Molecular weight range:	447.45

Structural formula:
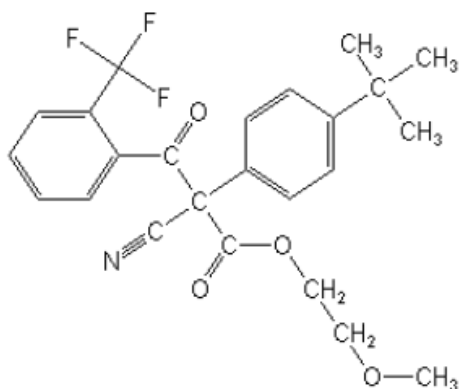
1.2   Composition of the substance

There is no FAO specification for this substance.

Current Annex VI entry: currently no Annex VI entry

Table 5 Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
cyflumetofen	≥975 g/kg (racemic)	≥975 g/kg (racemic)	

Table 6 Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

Technical cyflumetofen does not contain toxicologically or environmentally relevant impurities.

Table 7 Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Confidential				

1.2.1   Composition of test material

Most of the (eco)toxicological studies were performed with test material analysed for its purity/impurity profile and all batches analysed were within the technical specifications (DAR cyflumetofen, volume 4, confidential information).
One batch not analysed for its purity/impurity file was used for the study: Repeated dose 28-day oral toxicity study in rats. This batch was manufactured in the same way as the batches analysed for their purity/impurity profile. Because of the strong similarities between the batches analysed, the

profile for the batch used in the repeated dose 28-day oral toxicity study in rats is considered to be comparable to the batches tested for their purity/impurity profile.

No purity/impurity profile is available of the batch used for the absorption, distribution, excretion and metabolism (ADME) studies; however, the presence of impurities is of minor concern for this type of studies.

The batch used for the study: Repeated dose 2-weeks oral toxicity study in rats had a purity of 98%. There is no impurity file for this batch, however, this study is considered supplementary.

One impurity appeared not in all batches analysed and therefore an assessment was made on the toxicological relevance of this impurity showing that this impurity is of no toxicological concern.

Cyflumetofen is a racemic mixture. The potential preferential metabolism/degradation of each enantiomer in animals and the environment was not investigated in the studies available.

In the EFSA conclusion (2012) the following is stated: "The batches used in the toxicological studies support the agreed technical specification."

The recent performed chronic toxicity and carcinogenicity studies in rats and mice (Takahashi, 2013 and Yoshida, 2013) were also performed with a batch in agreement with the technical specification (Pluijmen, 2014).

In the EFSA conclusion (2012), it was stated that "the relevance of most of the impurities was not addressed". However, this is not in line with the information provided in the DAR of cyflumetofen, volume 4, confidential information (addendum October 2011) and the results of the Expert Consultations: Based on the reporting table of cyflumetofen (points I(5, 6 and 8)) the following is concluded by the Pesticides Peer Review Meeting: "A revised specification, removing impurities A-2, B-2, AB-9, AB-10, AB-12 specified at 1 g/kg." For impurity AB-13 the following is concluded (Expert consultation 2.6): "The impurity AB-13 has been adequately tested and no further toxicological investigations are needed."

Overall, the composition of the test material is considered acceptable for the prediction of the toxicity of cyflumetofen as placed on the market.

1.3   Physico-chemical properties

Table 8 Summary of physico - chemical properties

Property	Value (purity substance)	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101.3 kPa	White odourless solid (98.46% PAI[b]) Yellow solid with no characteristic odour (98.4% TGAI[c])	Lijima, 2001a (IIA 2.4.1/01) [a], Lijima, 2001b (IIA 2.4.1/02) [a], Baltussen, 2006a (IIA 2.4.1/03) [a], Lijima, 2001c (IIA 2.4.2/01) [a], Baltussen, 2006a (IIA 2.4.2/02) [a]	Visual observation

Property	Value (purity substance)	Reference	Comment (e.g. measured or estimated)
Melting/freezing point	77.9 – 81.7 °C (98.46%)	Lijima, 2002a (IIA 2.1.1/01)[a]	Measured
Boiling point	293 °C (98.46%)	Lijima, 2002b (IIA 2.1.2/01)[a]	Measured
Thermal stability	No decomposition observed up to 500°C (98.46 %)	Lijima, 2002b (IIA 2.1.3/01) [a], Lijima, 2002c (IIA 2.1.3/01) [a]	Measured
Density	1.229 g/cm^3 at 20°C (98.46 %)	Lijima, 2003a (IIA 2.2/01)[a]	Measured
Vapour pressure	< 5.9x10^{-6} Pa at 25 °C (98.4%)	Saka, 2002 (IIA 2.3.1/01) [a]	Measured
Surface tension	-	-	Not required, water solubility < 1 mg/L
Henry's law constant	< 9.4x10^{-2} Pa.m^3.mol^{-1}	Cardinaals, 2006a (IIA 2.3.2/01) [a]	Calculated
UV / VIS absorption (max.) incl ε	At 25 °C (98.46%): No maximum above 290 nm, but significant absorption does occur (ε > 10 L.mol^{-1}.cm^{-1}) at acidic and neutral conditions. At alkaline conditions cyflumetofen is insufficiently stable to conclude if absorption is of breakdown products or of cyflumetofen.	Lijima, 2002d (IIA 2.5.1.1/01)[a]	Measured
Water solubility	28 µg/L at 20 °C and pH 7 (98.46%). No pH dependence	Lijima, 2004a (IIA 2.8.1/01)[a]	Measured
Partition coefficient n-octanol/water	Log Pow = 4.3 at 25 °C (98.46%) No pH dependence	Lijima, 2003a (IIA 2.2/01)[a]	Measured
Flash point	-	-	Not required, melting point > 40°C
Flammability	Not highly flammable (98.0% TGAI)	Van der Baan-Treur, 2004a (IIA 2.11.1/01) [a]	Measured
Explosive properties	Not explosive (98.0% TGAI)	Van der Baan-Treur, 2004c (IIA 2.13/01) [a]	Statement
Self-ignition temperature	Auto-ignition at 320 °C (98.0% TGAI)	Van der Baan-Treur, 2004b (IIA 2.11.2/01) [a]	Measured

Property	Value (purity substance)	Reference	Comment (e.g. measured or estimated)
Oxidising properties	Not oxidising (98.0% TGAI)	Van der Baan-Treur, 2004d (IIA 2.15/01) [a]	Statement
Granulometry	No data		
Solubility in organic solvents	Solubility at 20 °C (98.46%): acetone: >500 g/L solvent dichloromethane: >500 g/L solvent ethyl acetate: >500 g/L solvent n-hexane: 5.16 g/L solution methanol: 98.7 g/L solution toluene: >500 g/L solvent	Lijima, 2003c (IIA 2.7/01)[a]	Measured
Dissociation constant	No dissociation expected in a relevant pH range.	-	-
Viscosity	-	-	Not required, substance is a solid at ambient conditions

[a] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Volume 3, Annex B.2; October 2010.

[b] PAI: Purified Active Ingredient

[c] TGAI: Technical Grade Active Ingredient

The above data refer to cyflumetofen. The data are obtained from the European Food Safety Authority (EFSA) conclusion on pesticide peer review (Conclusion on the peer review of the pesticide risk assessment of the active substance cyflumetofen; EFSA Journal 2012; 10(1):2504), except for the thermal stability and density data which were taken from the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Volume 3, Annex B.2; October 2010.

2   MANUFACTURE AND USES

2.1   Manufacture

Not relevant for this type of report.

2.2 Identified uses

Cyflumetofen is a specific acaricide and is used in both indoor and outdoor spray application to ornamental crops, nursery trees, perennial ornamentals and to public greens for the control of *Tetranychyus urticae* (red spider mite).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 9 Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flash point	Not required, melting point > 40°C		
Flammability; EC A.10	Not highly flammable	CLP requires Method N.1 as described in 33.2.1 of the UN RTDG [b], Manual of Tests and Criteria	Van der Baan-Treur, 2004a (IIA 2.11.1/01) [a]
Explosive properties; statement	Not explosive		Van der Baan-Treur, 2004c (IIA 2.13/01) [a]
Self-ignition temperature; EC A.15	Auto-ignition at 320 °C		Van der Baan-Treur, 2004b (IIA 2.11.2/01) [a]
Oxidising properties; statement	Not oxidising		Van der Baan-Treur, 2004d (IIA 2.15/01) [a]
Thermal stability; DTA/TGA (open vessel) Comparable to OECD 113	No thermal instability	No decomposition takes place up to the boiling point as the thermal analysis shows no endotherm that could be linked to decomposition prior to boiling.	Iijima K, 2002b (IIA 2.1.3/01) [a]
		Heating was continued up to 500 °C, but no sign of residue in the test vessel was observed, probably due to evaporation (it should be noted that the self-ignition temperature of cyflumetofen is 320 °C).	Iijima K, 2002c (IIA 2.1.3/01) [a]
Self-reactive substances and mixtures	Not a self-reactive substance	Following the criteria as presented in Appendix 6, Section 5.1 of the UN-Manual of Tests and Criteria	
Pyrophoric solids	Not pyrophoric	Self-ignition temperature above room temperature	
Self-heating substances and mixtures	Not a self-heating substance	Based on the melting point < 160 °C the substance needs not be considered for self-heating classification	
Substances and mixtures which in contact with water emit flammable gases	No emission of flammable gases in contact with water	The chemical structure of the substance does not contain metals or metalloids and experience in handling and use shows that the substance does not react with water	
Substance and mixtures corrosive to metals	-	No appropriate test design available for low water soluble solids	

[a] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Volume 3, Annex B.2; October 2010.

[b] UN Recommendations on the Transport of Dangerous Goods

3.1 Physical chemical properties

3.1.1 Summary and discussion of physical chemical properties

CLP Regulation:

Explosives: Not explosive.

Flammable solids: In the EC A.10 study a pre-test was considered sufficient as the test substance pile could not be ignited (Van der Baan-Treur, 2004a). Required for CLP is Method N.1 as described in 33.2.1 of the UN Recommendations on the Transport of Dangerous Goods (RTDG), introducing a wetted zone in the main test (relative to EC A.10) to distinguish between Category 1 and category 2 flammable solids. This difference is however not considered relevant for a substance concluded from the pre-test to be not a flammable solid.

Self-reactive substances: The chemical structure of the substance does not indicate any potential for self-reactivity. The screening procedures to conclude on self-reactive substances as presented in Appendix 6, Section 5.1 of the UN-MTC (Manual of Tests and Criteria) confirm that cyflumetofen does not contain any chemical groups associated with explosive (Table A6.1 of Appendix 6) or self-reactive (Table A6.2 of Appendix 6) properties.

Pyrophoric solids: As the self-ignition temperature for cyflumetofen is greater than the room (ambient) temperature, the substance is concluded not to be a pyrophoric substance.

Self-heating substances: Substances with a low melting point (< 160 °C) need not be considered for classification for this property since the melting process is endothermic and the substance-air surface is drastically reduced. As cyflumetofen is completely molten at 160 °C, it is concluded not to be a self-heating substance.

Substances which in contact with water emit flammable gases. The chemical structure of the substance does not contain metals or metalloids. Furthermore, experience in handling and use shows that the substance does not react with water: cyflumetofen is formulated into an aqueous Suspension Concentrate (SC) designed to be further diluted with water prior to application; emission of flammable gases has not been observed.

Oxidising solids: Not oxidising.

Corrosive to metals: Cyflumetofen is a solid that may become a liquid through dissolution in water or in a solvent. The adequate corrosive to metals testing procedure for these substances is complex and not explicitly described in the relevant UN-MTC test protocol (C.1 test for determining the corrosive properties of liquids and solids that may become liquids during transport). The ECHA Guidance on the application of the CLP criteria furthermore indicates that it needs to be further specified how such substances must be prepared (transformed into liquids) to be able to determine their corrosivity to metals; simple dilution of the solid substance in any quantity of water or other solvent will in no case lead to satisfactory testing of the substance for corrosion to metals. Cyflumetofen is not a strong acid or alkaline substance and has low water solubility; any potential consideration for classification is the presence of halogen in the molecule. Awaiting the availability of a relevant test design, cyflumetofen is considered as being not corrosive to metals.

3.1.2 Comparison with criteria

CLP Regulation: Cyflumetofen does not fulfil any of the criteria for classification for physical hazards deemed relevant for a solid substance. No experimental data are available for the corrosion to metals endpoint as no a relevant test design presently exists for solid substances.

3.1.3 Conclusions on classification and labelling

CLP Regulation: The technical substance need not to be classified for any of the physical hazard endpoints deemed relevant for a solid substance.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) evaluated the physical properties of cyflumetofen, based on the results of the standard test included in the CLH dossier. Technical grade cyflumetofen is not a flammable substance, does not self-ignite and has no oxidising or explosive properties. The chemical structure of the substance does not indicate any potential for self-reactivity. The substance does not react with water and the emission of flammable gases has not been observed. Cyflumetofen is not a strong acid or alkaline substance and has low water solubility, it is considered as being not corrosive to metals.

Comments received during public consultation

Two comments received from industry. Both were in support of the Dossier Submitter's (DS) proposal not to classify for physical hazards.

Assessment and comparison with the classification criteria

Cyflumetofen **does not fulfil any of the criteria for classification for physical hazards** deemed relevant for a solid substance.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Several toxicokinetic studies with cyflumetofen have been performed in rats (Nakamura, 2004c; Nakamura, 2004d; Ohyama, 2004a; Ohyama, 2004b; Nakamura, 2004e; Nakamura, 2004f; Hardwick, 2008). In addition a toxicokinetic study was performed in mice (Fabian 2011).

Toxicokinetics

The systemic absorption of 14C-cyflumetofen radiolabelled in the A- or B-ring (see Figure 1) and the plasma kinetics were investigated in male and female rats after a single oral dose of 3 or 250 mg/kg bw. The C_{max} of 0.95-1.4 mg eq/L was reached after 1 hour (low dose) and the C_{max} of 10-15 mg eq/L was reached after 2-4 hours (high dose). The slow phase elimination half-life was 14-17 hours (A-label) and 17-21 hours (B-label). The factor between AUC (systemic absorption) at low and high dose was 15-38, and much lower than the factor of 83 between dose levels, an indication that saturation occurred (possibly because the dosing formulation was a suspension). There were no important differences between kinetic parameters of males and females at low dose, but at high dose the plasma peak level and systemic absorption were higher in females, and the concentration remained longer near peak level. There were no important differences between kinetic parameters for A- and B-label, except the slow phase elimination half-life, which was longer for the radioactivity from the B-ring.

In mice the plasma kinetics were investigated after a single oral dose of 0, 50, 250, and 1000 mg/kg bw of the B-ring label to male and female CD-1 mice (Fabian 2011). The parameters derived from the plasma kinetics in mice are presented in Table 10.

Table 10 Parameters from a toxicokinetic study in mice (Fabian 2011)

Sex	Dose [mg/kg bw]	c_{max} [µg Eq/g]	T_{max} [h]	initial half life [h]	terminal half life [h]	AUC $_{0 \to \infty}$ [µg Eq *h/g]
male	1000	65.40	0.5	1.38	54.89	716.83
	500	43.28	0.5	2.82	53.16	490.41
	250	27.12	0.5	0.70	60.07	252.99
	50	10.61	0.5	2.02	39.79	89.15
female	1000	103.83; 36.87	0.5, 8	1.02	35.22	1106.46
	500	65.32; 27.12	1, 8	2.25	43.87	771.06
	250	34.27; 46.54	0.5, 2	2.30	41.33	365.90
	50	51.84; 5.96	1, 8	0.42	22.04	176.74

[14]C BAS 9210 I (Cyflumetofen) was rapidly absorbed through gastrointestinal tract of male and female mice with first C_{max} at 0.5h or 1 h. Oral administration of [14]C_BAS 9210 I (Cyflumetofen) resulted in higher absorption in females compared to males. Additionally, the time course of

radioactivity differed between both sexes. Females showed a second C_{max} at 2 or 8 hours after administration. Higher absorption of the test substance and an enterohepatic recirculation in females led to higher internal doses (represented by ($AUC_{0\to\infty}$) values) compared to males. In both sexes the internal dose increased sublinear but continuously with increasing oral dose over the dose range tested.

Absorption

Absorption of radioactivity was investigated in male and female rats after a single oral dose of 3 or 250 mg/kg bw 14C-cyflumetofen radiolabelled in the A- or B-ring. Radioactivity recovered after 72 hours in urine represented 58-67% AR (low dose) and 14-26% AR (high dose), and that in faeces 25-33% AR (low dose) and 68-80% AR (high dose). Retention of radioactivity in the tissues and carcass was 0.9-2.5% AR (low dose) and 0.4-0.8% AR (high dose). Based on the radioactivity recovered from urine, tissues and residual carcass, absorption by rats given a single oral dose of 3 and 250 mg/kg bw was 61-67% and 15-26% respectively. There were no remarkable differences between radiolabels. Excretion of radioactivity with urine and absorption was higher for female than for male rats, irrespective of dose level.

A separate study was performed using bile duct cannulated rats at the same dose levels with A- and B-ring 14C-cyflumetofen. Based on the radioactivity recovered from urine, bile and tissues and residual carcass, oral absorption by rats given a single oral dose of 3 and 250 mg/kg bw was 68-78% and 35-46% respectively, without remarkable differences between radiolabels and sexes.

Dermal absorption of cyflumetofen was also evaluated. An *in vitro* study with cyflumetofen was performed with human dermatomed skin membranes. The test solutions were prepared using cyflumetofen (OK-5101) dissolved in acetonitrile. Skin membranes were exposed to 0.2 or 200 g/L, for 8 hours, during which the donor chamber was occluded (application volume: 6.4 µl/0.64 cm^2= 10 µl/cm^2). The results show that the actual dermal absorption into the receptor fluid is low: about 0.6% is absorbed 24 hours after application of a low or high dose (8 hour exposure). However, there is a relatively large dermal depot (skin and tape strips) of about 20 and 27% for the low and high dose, respectively. Therefore, the potentially absorbed dose is relatively high, but is almost completely determined by the dermal depot. Probably part of the dermal depot will be lost by desquamation, but this cannot be quantified. As worst-case it is therefore concluded that the dermal absorption of cyflumetofen is 21% for the low dose and 28% for the high dose (rounded values). This should furthermore be considered as a worst-case estimate because acetonitrile probably enhances the dermal absorption of cyflumetofen. Finally also the physical-chemical properties of cyflumetofen support a relatively low dermal absorption (molecular weight is 447.45 and log Pow is 4.3), but based on the available study data the dermal absorption values of 21 and 28% (for the low and high dose, respectively) can be used for the risk assessment as worst-case.

Distribution

Tissue distribution of radioactivity was investigated in male and female rats at T_{max} and 24 hours (first study) and at 72 hours (second study) after a single oral dose of 3 or 250 mg/kg bw 14C-cyflumetofen radiolabelled in the A- or B-ring.

For all time points, and irrespective of label and sex, the highest radioactivity concentrations were found in the liver, followed by kidney. After 1-2 hours and 24 hours the radioactivity concentrations in kidney were on average a factor of 4 higher than those in plasma, and those in liver a factor of 12. The radioactivity concentrations in other tissues and organs were below those in plasma at these time points.

After 72 hours, the highest radioactivity concentrations were found in liver, followed by kidney. In rats dosed with A-label-14C-cyflumetofen, radioactivity concentrations in erythrocytes (high dose only), adipose tissue, adrenal gland and bone marrow were higher than in plasma. In rats dosed with B-label-14C-cyflumetofen, radioactivity concentrations in erythrocytes and adrenal gland (high dose only) were higher than in plasma. There were no remarkable differences between results of male and female rats. Concentrations in tissues and organs of rats treated with the high dose were 18-101 times higher (mean 53 times) than those of rats treated at the low dose. Radioactivity concentrations in most tissues and organs of rats treated with B-label-14C-cyflumetofen were slightly higher (on average 1.6 times) than in those of rats treated with A-label-14C-cyflumetofen. An exception was the radioactivity in liver and bone marrow, which was lower in rats treated with B-label-14C-cyflumetofen than in rats treated with A-label-14C-cyflumetofen.

The half-life for elimination of radioactivity from plasma ranged between 8.9 and 15 hours. The half-life for elimination of radioactivity from blood, kidney and liver was slightly higher than from plasma ranges were 10-19, 11-17 and 13-19 hours respectively. Elimination from adipose tissue (half-life 19-43 hours) and bone marrow (half-life 14-30 hours) was slower than from plasma and other tissues. The elimination pattern of radioactivity from most tissues (but not liver) appeared to be bi-phasic at the lower dose, in particular in female rats.

Tissue distribution of radioactivity was also investigated in female rats that had received 14 daily oral doses of 3 mg/kg bw 14C-cyflumetofen radiolabelled in the B-ring, at 24 hours after the 4th and 7th dose and at 2, 24 and 72 hours after the last (14th) dose. At all samplings, radioactivity concentrations were highest (and exceeded those in plasma) in liver, followed by kidney and (except 2 hours after the last dose) erythrocytes. Already after 4 daily doses equilibrium had been achieved, and no remarkable accumulation occurred in any of the tissues and organs investigated. The radioactivity concentration in tissues and organs at 24 and 72 hours, respectively, after the 14th dose were on average 21% and 11% of those after 2 hours, with the slowest depletion occurring in erythrocytes (34% and 25%) and skin (40% and 30%).

Metabolism

The metabolite distribution was investigated in samples of urine and faeces from male and female rats collected during 72 hours after a single oral dose of 3 or 250 mg/kg bw 14C-cyflumetofen radiolabelled in the A- or B-ring.

Individual unidentified fractions in urine and faeces each represented at the most 3.5% AR. Unchanged parent cyflumetofen was not detected in urine.

Major identified compounds in urine were:

-        B-1 (up to 9.7% AR),

-        the thiolactic acid conjugate of B-1 (up to 20% AR),

-        the mercapturic acid conjugate of B-1 (up to 14% AR),

-        A-18 (up to 34% AR) and

-        A-21 (up to 21% AR).

Also identified were:

-        AB-3 (up to 8.8% AR),

-        AB-2 (up to 4.3% AR),

- A-20 (up to 3.9% AR) and

- the glucuronic acid conjugate of A-6 (up to 1.9% AR).

AB-3 was found at much higher levels in urine of female rats than in urine of male rats, which is likely to be due to selective excretion by female rats, since AB-3 was found in bile of both male and female rats at comparable levels (see below).

The higher levels of A-18 in urine of female rats could be explained by the fact that the pathway which converts A-18 into A-21 and A-20 was more active in female rats than in male rats.

Non-extractable radioactivity in faeces samples represented <4.0% of the administered radioactive dose. Unchanged parent cyflumetofen was found in faeces at levels up to 4.3% AR and up to 66% AR at low and high dose respectively. The difference between dose levels may be attributed to saturation of absorption.

A major metabolite in faeces was:

- B-1 (up to 17% AR).

Also identified in faeces were:

- A-12 (up to 1.9% AR) and

- A-20 (up to 3.2% AR).

The metabolite distribution was also investigated in bile of male and female rats at 48 hours after a single oral dose of 3 or 250 mg/kg bw 14C-cyflumetofen radiolabelled in the A- or B-ring.

Unchanged cyflumetofen was only detected in bile at or near the LOQ (0.03-0.08% AR) in the low dose B-ring sample of male and female rats, but not in the corresponding A-ring sample, nor in any high dose sample.

Major identified compounds in bile were:

- the glucuronic acid conjugate of AB-3 (up to 8.2% AR)

- the glucuronic acid conjugate of AB-1 (up to 13% AR).

Also identified at levels >1% AR were:

- AB-2 (up to 3.2% AR),

- the glutathione conjugate of B-1 (up to 2.6% AR),

- the glucuronic acid conjugate of A-6 (up to 3.7% AR) and

- the glucuronic acid conjugate A-22 (up to 3.8% AR).

The metabolite distribution in samples of urine and faeces was also investigated in urine and faeces from female rats collected during 72 hours after the last of 14 daily oral doses of 3 mg/kg bw 14C-cyflumetofen radiolabelled in the B-ring. The only reference standards used for co-chromatography during HPLC were cyflumetofen and 2-trifluoro-methyl benzoic acid (B-1). Unchanged parent cyflumetofen was not detected in urine (which contained 64% of the radioactivity administered in

the last dose), whilst B-1 was found at 13% of the radioactivity administered in the last dose. The urine contained 9 other non-identified fractions (0.7-12% of the radioactivity administered in the last dose). Radioactivity in faeces extract (51% of the radioactivity administered in the last dose) consisted mainly of parent cyflumetofen (25% of the radioactivity administered in the last dose) and B-1 (22% of the radioactivity administered in the last dose). The design of this study involved successive daily dosing with radioactive 14C-cyflumetofen, rather than a single radioactive dose after 13 daily doses with non-radiolabelled cyflumetofen, and this prevents full comparison of its results with those from the studies with single oral dose administration. However, about half of the radioactivity excreted from faeces represented parent cyflumetofen, whereas following a single oral dose of B-label parent 14C-cyflumetofen (2.5% AR) represented only a minor portion of the radioactivity in faeces extract (23-29% AR). This suggests that saturation of absorption occurs following repeated dosing.

No information is available as to whether the known reprotoxic substance methoxyethanol (CAS 109-86-4) can be formed by hydrolysis of the ester. Methoxyethanol was not identified as metabolite in the ADME studies.
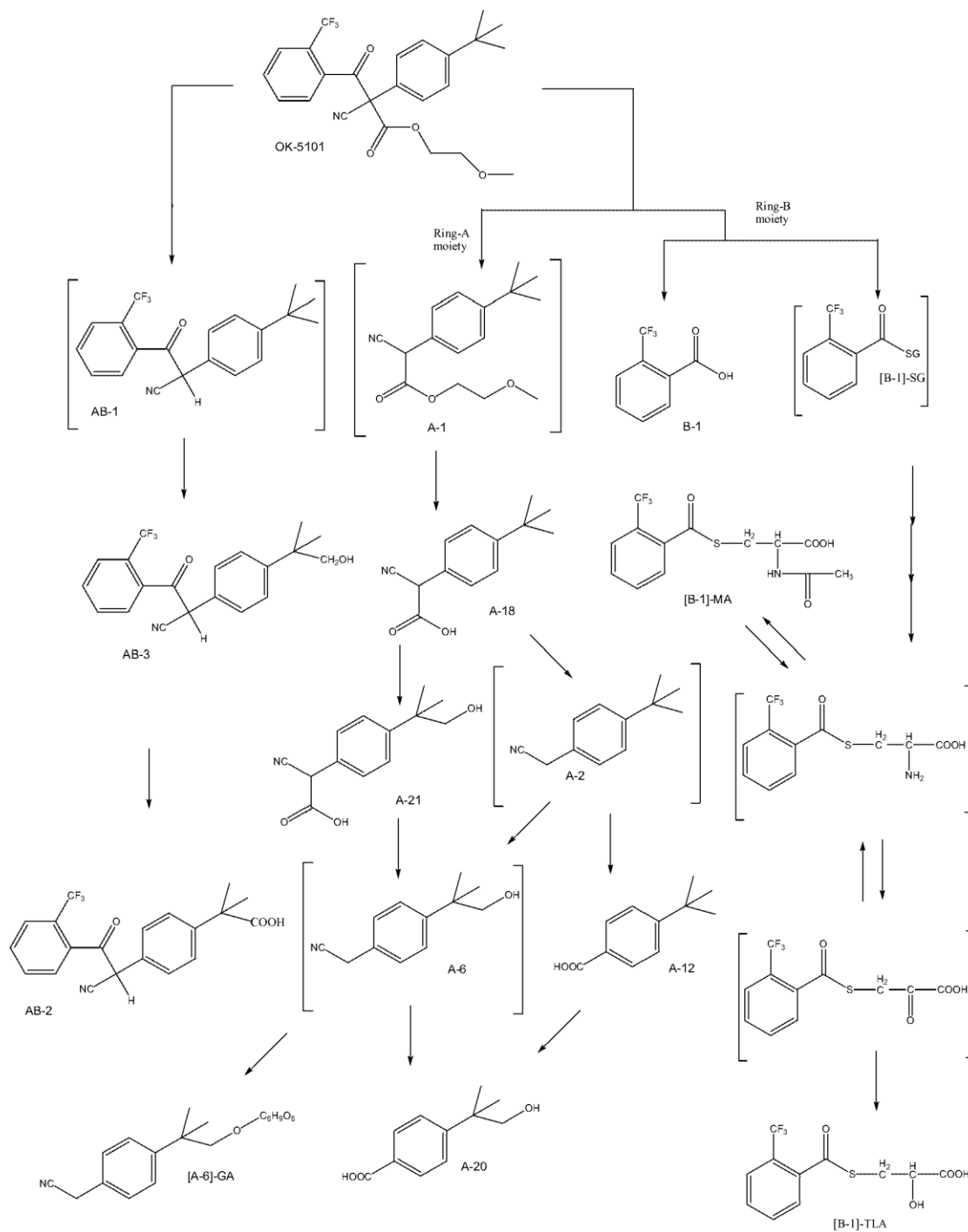
Figure 1   Proposed metabolic pathway of cyflumetofen (OK-5101)

Excretion

Excretion of radioactivity was investigated in male and female rats after a single oral dose of 3 or 250 mg/kg bw 14C-cyflumetofen radio labelled in the A- or B-ring.

The major pathway of excretion of radioactivity was the urine for low dose rats, but the faeces for high dose rats. Radioactivity recovered after 72 hours in urine represented 58-67% AR (low dose) and 14-26% AR (high dose), and that in faeces 25-33% AR (low dose) and 68-80% AR (high dose). There were no remarkable differences between radiolabels. Excretion of radioactivity with urine was higher for female than for male rats, irrespective of dose level. Excretion of radioactivity in expired air was insignificant (<0.008% AR after 24 hours in a preliminary study using the same dose levels and radiolabels).

In a separate study performed using bile duct cannulated rats at the same dose levels with A- and B-ring 14C-cyflumetofen, excretion of radioactivity after 48 hours in bile of male rats (29-37% AR) exceeded that of female rats (18-25% AR), without remarkable differences between radiolabels and dose levels. Comparison of results from studies with cannulated and non-cannulated rats showed that at the low dose (but not at the high dose) radioactivity excreted in bile was re-absorbed from the intestinal tract and then excreted in urine.

Elimination was also investigated in female rats that had received 14 daily oral doses of 3 mg/kg bw 14C-cyflumetofen radiolabelled in the B-ring. 72 Hours after the 14th dose, overall recovery of radioactivity was 132% (based on the radioactivity in the last dose) or 9.1% (based on the total radioactivity in the 14 doses). Approximately equal amounts of radioactivity were excreted with urine and faeces (totalling 123% based on the radioactivity in the last dose and 8.4% based on the total radioactivity in the 14 doses). The design of this study involved successive daily dosing with radioactive 14C-cyflumetofen, rather than a single radioactive dose after 14 daily doses with non-radiolabelled 14C-cyflumetofen, and this prevents full comparison of its results with those from the studies with single oral dose administration. However the fact that equal amounts of radioactivity were excreted in urine and faeces, whereas following a single oral dose of B-label the radioactivity in faeces (25-33% AR) was much lower than in urine (59-67%AR), suggests that saturation of absorption occurs following repeated dosing.

4.1.2   Human information

No data

4.1.3   Summary and discussion on toxicokinetics

Rats

The oral absorption of cyflumetofen is 61-67% or 15-26% after single low dose (3 mg/kg bw) or high dose (250 mg/kg bw), respectively, based on radiolabel recovered from urine, tissues and residual carcasses measured 72 hours after administration. In bile duct cannulated rats oral absorption was 68-78% or 35-46% at 3 mg/kg bw and 250 mg/kg bw, respectively. The radiolabel was mainly distributed to liver and kidneys. Cyflumetofen is extensively metabolized including glucuronic acid conjugation, glutathione conjugation, mercapturic acid conjugation and thiolactic acid conjugation. Excretion was rapid with 80% or more of the administered radiolabel within 72 hours, irrespective of sex or dosing regimen. Most radiolabel was excreted via urine at low dose, whereas after high dose administration most radiolabel was excreted in faeces. No differences in kinetic parameters between sexes was observed at low dose, whereas at high dose females showed higher plasma peak levels and the concentration remained longer near peak level. The observation of significant increased amount of parent compound excreted via faeces after repeated dosing compared to single dosing suggests saturation of absorption following repeated dosing.

Mice

Cyflumetofen was rapidly resorbed through gastrointestinal tract of male and female mice with first T_{max} of 0.5h or 1 h. Oral administration resulted in higher absorption in females compared to males. The time course of radioactivity differed between the sexes. Females showed a second T_{max} of 2 or 8 hours after administration. Higher plasma concentrations and the observed enterohepatic recirculation in females resulted in higher internal doses shown by $AUC_{0\to\infty}$ values (1.4 – 2.0 fold higher in females compared to males). AUC values increased sublinear but continuously with increasing dose.

4.2   Acute toxicity

Table 11 Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
OECD 420 (oral toxicity), rat	LD50 > 2000 mg/kg bw	Fixed dose procedure	[a]Moore, E.L., 2003a
OECD 402 (dermal toxicity), rat	LD50 > 5000 mg/kg bw	Limit test	[a]Moore, E.L., 2003b
OECD 403 (inhalation toxicity), rat	LC50 > 2.65 mg/L	Limit test	[a]Bowden, A.M., 2003

[a]As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.2.1   Non-human information

4.2.1.1 Acute toxicity: oral

Reference	:	Moore, E.L., 2003a	exposure	: Once by gavage
type of study	:	Acute oral toxicity study	doses	: 2000 mg/kg bw
year of execution	:	2003	vehicle	: 5% Gum Arabic and 4% Tween 80
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	: Yes
Route	:	Oral	guideline	: According to OECD 420
Species	:	Rat, Wistar Han	acceptability	: Acceptable
group size	:	5f (step 1 and step 2)	**LD$_{50}$**	: >2000 mg/kg bw

Cyflumetofen (purity 98.0%) was tested in an acute oral toxicity study with female rats at a dose level of 2000 mg/kg bw (fixed dose procedure). No mortality occurred at 2000 mg/kg bw. In one female loose faeces was noted 5 hours after dosing, resolving completely by day 2. No treatment related findings were observed for the remaining animals. The acute oral LD_{50} of cyflumetofen was found to be greater than 2000 mg/kg bw in female rats.

4.2.1.2 Acute toxicity: inhalation

reference	:	Bowden, A.M., 2003	exposure	: 4 hours; nose-only
type of study	:	Acute inhalation toxicity study	doses	: 17.9 mg/L (nominal concentration); 2.65 mg/L (actual concentration); MMAD 5.0 μm with GSD 2.6.
year of execution	:	2002	vehicle	: None
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	: Yes
route	:	Inhalation	guideline	: According to OECD 403
species	:	Rat, Wistar Han	acceptability	: Acceptable
group size	:	5/sex	**LC$_{50}$**	: >2.65 mg/L

Cyflumetofen (purity 98.0%) was tested in an acute inhalation toxicity study with rats at a dose level of 2.65 mg/L (actual concentration, MMAD 5.0 μm with GSD 2.6; maximum attainable concentration). No mortality occurred at a concentration of 2.65 mg/L. During and after exposure exaggerated breathing was noted 15 minutes from the start of exposure till day 1 of observation. Immediately following exposure brown staining around snout/jaws was also observed. A slightly reduced mean body weight gain was observed for male and female rats during the first week. During the second week the mean body weight gain was normal. The acute LC_{50} of cyflumetofen in male and female rats was found to be greater than 2.65 mg/L (maximum attainable concentration).

4.2.1.3 Acute toxicity: dermal

reference	:	Moore, E.L., 2003b	exposure	:	24 hours on a skin area of about 25 cm^2 (semi-occlusive exposure).
type of study	:	Acute dermal toxicity study	doses	:	5000 mg/kg bw
year of execution	:	2002	vehicle	:	1% w/v aqueous methylcellulose
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 402
species	:	Rat, Wistar Han	acceptability	:	Acceptable
group size	:	5/sex	**LD_{50}**	:	>5000 mg/kg bw

Cyflumetofen (purity 98.0%) was tested in an acute dermal toxicity study with rats at a dose level of 5000 mg/kg bw/day. No mortality was observed at 5000 mg/kg bw. No treatment related findings were observed. The acute dermal LD_{50} of cyflumetofen in male and female rats was found to be greater than 5000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

No data.

4.2.2 Human information

No human data available.

4.2.3 Summary and discussion of acute toxicity

No mortality was observed in acute oral, dermal, and inhalation studies in rats at the limit dose.

4.2.4 Comparison with criteria

Classification is required when 50% or more of the test animal die at or below 2000 mg/kg bw (oral and dermal) or 5 mg/L or maximum attainable concentration (inhalation of dust). No such effect occurred. Therefore, cyflumetofen does not meet the criteria for classification based on the acute toxicity studies.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity is required.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS summarised a single GLP OECD guideline-compliant acute toxicity study with (Han Wistar) rats for each route (oral, dermal and inhalation).

The oral acute toxicity study (Moore, 2003a) was conducted in accordance with OECD TG 420 using female rats only at 2000 mg/kg bw (fixed dose procedure). Since there was no mortality at that dose, the DS concluded that the criteria in the CLP Regulation were not met and proposed no classification for acute oral toxicity.

The acute dermal toxicity study was conducted in accordance with OECD TG 402 using rats of both sexes (Moore, 2003b). Since there was no mortality at 5000 mg/kg bw, the DS concluded that the criteria in the CLP Regulation were not met and proposed no classification for acute dermal toxicity.

The acute inhalation toxicity study (Bowden, 2003), was conducted in accordance with OECD TG 403 using rats of both sexes at a dose level of 2.65 mg/L (MMAD 5.0 μm; mass median aerodynamic diameter). Clinical signs immediately following exposure included exaggerated breathing and brown staining around snout/jaws, as well as a slightly reduced mean body weight gain (extent not shown) during the first week. Although the maximum attainable concentration used in the study was lower than 5.0 mg/L (the threshold for classification for acute inhalation toxicity for dusts and mists), there was no mortality at the tested dose. The DS concluded that the criteria in the CLP Regulation were not met and proposed no classification for acute inhalation toxicity.

Comments received during public consultation

Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal for no classification for acute toxicity.

Assessment and comparison with the classification criteria

Acute Oral Toxicity

The oral LD_{50} was > 2000 mg/kg bw in female rats in a guideline compliant and GLP study. Male rats were not tested. According to CLP Regulation, LD_{50} values for acute oral toxicity > 2000 mg/kg bw do not warrant classification. The RAC is in agreement with the DS, **cyflumatofen does not meet the criteria for classification and is therefore not classified for acute oral toxicity**.

Acute Inhalation Toxicity

An inhalation 4 h LC_{50} of > 2.65 mg/L (maximum attainable concentration) was derived from a guideline compliant and GLP study conducted in male and female rats. According to CLP Regulation, LC_{50} values for acute inhalation > 5 mg/L or maximum attainable concentration for dust/mist do not warrant classification. The RAC is in agreement with the DS. **No classification for acute inhalation is warranted for cyflumetofen**.

Acute Dermal Toxicity

Results from a guideline compliant and GLP study in rats showed no signs of toxicity or mortality at 5000 mg/kg bw. According to CLP Regulation, LD_{50} values for acute dermal toxicity > 2000 mg/kg bw do not warrant classification. The RAC is in agreement with the DS. **No classification for acute dermal toxicity is warranted for cyflumetofen**.

4.3   Specific target organ toxicity – single exposure (STOT SE)

4.3.1   Summary and discussion of Specific target organ toxicity – single exposure

In the acute toxicity studies as summarised in chapter 4.2.1 and 4.12.1.1 (acute neurotoxicity) no specific effects on target organs were observed.

4.3.2   Comparison with criteria

The substance does not meet the criteria for classification.

4.3.3   Conclusions on classification and labelling

No classification is needed.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS noted that in the acute toxicity studies and the acute neurotoxicity study (Buesen, 2010), no specific effects on target organs were observed, and hence the substance did not meet the criteria for classification for STOT SE 1 or 2. The DS also noted that there was no indication of adverse effects on the respiratory tract, and therefore no classification for respiratory tract irritation (STOT SE 3) was proposed.

Comments received during public consultation

Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal for no classification for STOT SE.

Assessment and comparison with the classification criteria

RAC agreed with the dossier submitter that **no classification for STOT SE is warranted.**

<div style="border:1px solid black; height:60px;"></div>

4.4   Irritation

4.4.1   Skin irritation

Table 12 Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
OECD 404	**Not irritating to skin**		**[a]Rees, P.B., 2003a**

[a]As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.4.1.1 Non-human information

reference	:	Rees, P.B., 2003a	exposure	:	4 hours, semi-occlusive, application area 6.25 cm²
type of study	:	Skin irritation study	doses	:	0.5 g
year of execution	:	2003	vehicle	:	none (treatment site wetted with 0.5 mL water)
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 404
species	:	Rabbit, New Zealand White	acceptability	:	Acceptable
group size	:	3 males	**Effect**	:	Not skin irritating

In a well performed skin irritation study, 3 male rabbits were exposed during 4 h to cyflumetofen (purity 98.0%). No signs of irritation were observed at all time-points (0-72 h).

4.4.1.2 Human information

No human data available.

4.4.1.3 Summary and discussion of skin irritation

In a well performed skin irritation study with male rabbits, no signs of irritation were observed.

4.4.1.4 Comparison with criteria

Classification is required when a mean score at or above 2.3 is observed for erythema/eschar or for oedema from gradings at 24, 48 and 72 hours in 2 or more out of 3 animals.
According to the guidance such score is required in at least 4 animals if the test is performed with 6 animals. Classification is also required if persistent effects are observed or very definite positive effects. No such effects were observed. The substance does not meet the criteria for classification.

4.4.1.5 Conclusions on classification and labelling

No classification is needed.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS summarised a single GLP and OECD TG 404 compliant skin irritation study using 3 male New Zealand White (NZW) rabbits, in which no signs of irritation were observed (Rees, 2003a). Rabbits were exposed to cyflumetofen using semi-occlusive dressing for 4 hours. No signs of irritation were observed at any time-point (0-24-48-72 h). The DS concluded that the criteria for classification according to the CLP Regulation were not met and no classification was proposed for skin corrosion/irritation.

Comments received during public consultation

Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal for no classification for skin corrosion/irritation.

Assessment and comparison with the classification criteria

Classification is required when a mean score per animal at or above 2.3 is observed for erythema/eschar or for oedema from grading at 24, 48 and 72 hours in 2 or more out of 3 animals. No such effects were observed for cyflumetofen. RAC agrees with the DS that the substance **does not meet the criteria for classification of skin corrosion/irritation**.

4.4.2 Eye irritation

Table 13 Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
OECD 405	Not irritating to the eyes		[a]Rees, P.B., 2003b

[a] As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.4.2.1 Non-human information

reference	:	Rees, P.B., 2003b	exposure	: single instillation in conjunctival sac
type of study	:	Acute eye irritation study	doses	: 0.1 mL (92 mg)
year of execution	:	2003	vehicle	: None
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	: Yes
route	:	Ocular	guideline	: According to OECD 405
species	:	Rabbit, New Zealand White	acceptability	: Acceptable
group size	:	3f (and 1f in preliminary test)	**Effect**	: Not eye irritating

In a well performed eye irritation study, rabbits received a single instillation of cyflumetofen (purity 98.0%) in the eye. In two animals very slight discharge was seen one hour after instillation. Redness of the conjunctiva was observed till day 15. Treated eyes were normal on day 22.

Table 14 Results of the eye irritation test

Time	Cornea			Iris			Conjunctiva Redness			Chemosis		
Animal number	31	36	38	31	36	38	31	36	38	31	36	38
After 1 hour	0	0	0	0	0	0	1	1	1	0	0	0
after 24 hours	0	0	0	0	0	0	1	2	0	0	0	0
after 48 hours	0	0	0	0	0	0	1	1	1	0	0	0
after 72 hours	0	0	0	0	0	0	0	1	1	0	0	0
after 8 days	0	0	0	0	0	0	1	1	1	0	0	0
after 15 days	0	0	0	0	0	0	0	0	1	0	0	0
after 22 days	0	0	0	0	0	0	0	0	0	0	0	0
mean scores 24-72h	0	0	0	0	0	0	0.67	1.3	0.67	0	0	0

4.4.2.2 Human information

No human data available.

4.4.2.3 Summary and discussion of eye irritation

In a well performed eye irritation study, two rabbits showed very slight discharge one hour after instillation. Redness of the conjunctiva was observed till day 15. Treated eyes were normal on day 22.

4.4.2.4 Comparison with criteria

Classification as eye irritant is required when a mean score at or above 1 (corneal opacity or iritis) or 2 (conjunctival redness or conjunctival oedema) is observed from gradings at 24, 48 and 72 hours in 2 or more out of 3 animals.
No such effects were observed. The substance does not meet the criteria for classification.

4.4.2.5 Conclusions on classification and labelling

No classification is needed.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS summarised a single GLP and OECD TG 405 compliant eye damage/irritation study using 3 female NZW rabbits (Rees, 2003b). Redness of the conjunctiva was observed following instillation until day 15, but the mean scores over 24-72 h after exposure were < 2, and all treated eyes were normal by day 22 (table 14, CLH report). Cornea, iris and chemosis scores were 0 throughout the study period. The criteria for classification according to the CLP Regulation were not met and no classification was proposed for eye damage/irritation by the DS.

> **Comments received during public consultation**
>
> Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal for no classification for eye damage/irritation.
>
> **Assessment and comparison with the classification criteria**
>
> Classification as an eye irritant is required when a mean Draize score at or above 1 (corneal opacity or iritis) or 2 (conjunctival redness or conjunctival oedema) is observed from gradings at 24, 48 and 72 hours following installation of the test substance in at least 2 out of 3 animals.
>
> No such effects for cyflumetofen were observed. RAC agrees with the DS that the substance **does not meet the criteria for classification for serious eye damage/irritation**.

4.4.3 Respiratory tract irritation

4.4.3.1 Non-human information

In the animal studies available there are no indications that the substance has adverse effects on the respiratory tract.

4.4.3.2 Human information

No human data available.

4.4.3.3 Summary and discussion of respiratory tract irritation

There are no indications that the substance has adverse effects on the respiratory tract.

4.4.3.4 Comparison with criteria

The substance does not meet the criteria for classification.

4.4.3.5 Conclusions on classification and labelling

No classification is needed.

4.5 Corrosivity

Table 15 Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
OECD 404	Not irritating to skin		[a]Rees, P.B., 2003a

ᵃ As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.5.1   Non-human information

No corrosive properties were observed in a skin irritation study (see 4.4.1).

4.5.2   Human information

No human data available.

4.5.3   Summary and discussion of corrosivity

No corrosive properties were observed in a skin irritation study.

4.5.4   Comparison with criteria

The substance does not meet the criteria for classification.

4.5.5   Conclusions on classification and labelling

No classification is needed.

4.6   Sensitisation

4.6.1   Skin sensitisation

Table 16 Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
OECD 406; Maximisation test	Sensitizing to skin		Hooiveld, M.J.J., 2003a

ᵃ As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.6.1.1 Non-human information

reference	:	Hooiveld, M.J.J., 2003a	exposure	:	Intradermal and topical induction, topical challenge (occlusive)
type of study	:	Skin sensitisation study (M&K)	doses	:	1% intradermal induction 50% topical induction 50% challenge
year of execution	:	2003	vehicle	:	corn oil
test substance	:	OK-5101 (batch 01H1; purity 98.0%)	GLP statement	:	Yes
route	:	Dermal	guideline	:	According to OECD 406
species	:	Guinea pig, Dunkin Hartley	acceptability	:	Acceptable
group size	:	5f controls 10f test animals	**Effect**	:	Skin sensitising

Cyflumetofen (purity 98.0%) was tested in a maximization test. Dose levels were based on a preliminary irritation test using 0.1, 0.2, 0.5 and 1% cyflumetofen for intradermal injection and 5, 10, 20 and 50% cyflumetofen for topical exposure. The highest irritating dose for intradermal injection was 1% (erythema 1) and for topical application 50%. As no irritation was seen at the

latter, the site was pre-treated with 10% SDS 24 hours before topical induction. The highest non-irritant dose for challenge was considered to be 50% cyflumetofen.

Intradermal induction with 1% cyflumetofen resulted in slight to well-defined erythema and after topical induction with 50% cyflumetofen moderate erythema was observed. After topical challenge with 50% cyflumetofen, well-defined to moderate erythema in 10/10 females was observed after 24 hours and 48 hours. Scabs were noted in one and two animals after 24 and 48 hours, respectively; scaliness was seen in all females at 48 hours. Topical challenge in control animals did not induce any dermal reaction. No mortality, symptoms of systemic toxicity or effect on body weight was noted.

4.6.1.2 Human information

No human data available.

4.6.1.3 Summary and discussion of skin sensitisation

In a well performed maximisation test, all substance treated animals showed a positive effect.

4.6.1.4 Comparison with criteria

When more than 30% of the animals show a sensitizing effect in the maximisation test, the substance should be labelled as skin sensitizing. The results in the maximisation test fulfil the criteria as a sensitizing effect was observed in 100% of the animals. Further, sub-categorization in category 1A is required when more than 60% of the animals react after an intradermal induction dose at or below 1%. This criterion is also fulfilled.
There is no need to set a Specific Concentration Limit (SCL) for the substance:
Although the substance scored a high percentage of sensitized animals (100%), the intradermal induction concentration used in the study was 1%. Therefore, there is no evidence that the substance is an extreme sensitizer. Thus the compound is considered a strong sensitizing substance not needing a SCL.

4.6.1.5 Conclusions on classification and labelling

According to the criteria mentioned in the 'Guidance to Regulation No 1272/2008 on CLP' the substance should be classified for skin sensitisation, category 1A: H317 May cause an allergic skin reaction.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised a single "well performed" GLP and OECD TG 406 compliant Guinea Pig Maximisation Test (GPMT) using 10 female Dunkin Hartley guinea pigs, with 5 female animals as controls (Hooiveld, 2003a). The concentrations of cyflumetofen used were 1% for intradermal induction, 50% for topical induction (preceded by pre-treatment with 10% SDS for 24 h) and 50% for the challenge. After topical challenge with 50%

cyflumetofen, well-defined to moderate erythema was observed in 10/10 test animals after 24 and 48 hours, but no dermal reactions were seen in control animals. Since > 60% (100%) of treated animals responded to the treatment, and the intradermal induction concentration used in the study was 1%, the DS considered that cyflumetofen met the criteria for classification as Skin Sens. 1A. However, no SCL was proposed due to lack of evidence.

Comments received during public consultation

Three MSCAs agreed with the proposed classification. One MSCA considered the setting of specific concentration limits because of the large sensitisation response. However, the MSCA agreed that the available data was not sufficient to allow an SCL to be set. Also, since the substance had such a high number of sensitized animals (100%) with an intradermal induction concentration of 1%, there was also no evidence that the substance was not an extreme sensitiser. It is quite possible that if the testing had been done with an intradermal induction < 0.1% cyflumetofen could result in positive reactions in > 60% of the animals.

Two comments were received from industry addressing this endpoint. One agreed with the DS classification proposal. The DS assumed that the statement in the other industry comment that they "agree with the dossier submitter's proposal not to classify for skin sensitisation" contained a typing error and that in fact they also agreed with the proposed classification.

Assessment and comparison with the classification criteria

According to Annex I, Section 3.4.2.2.1.2 of CLP, where data are sufficient, a refined evaluation on the basis of the occurrence or potency of the sensitising effect can allow the allocation of skin sensitisers into sub-category 1A (high frequency of occurrence, strong sensitisers), or sub-category 1B (a low to moderate frequency of occurrence, low to moderate potency).

The criteria for classification in category 1A for skin sensitisation on the basis of the M&K GPMT are as follows: If a test substance is present at > 0.1 % to ≤ 1 % for intradermal induction and the incidence of sensitisation is ≥ 60%.

Cyflumetofen clearly meets the criteria for sub-category 1A.

The case for specifying an SCL is based on the fact that this substance appears to be a very strong sensitiser. There is an uncertainty as regards whether the GCL is sufficient or if an SCL would be more advisable. The criteria are quite clear but data is lacking for cyflumetofen which was only tested down to an intradermal induction of 1%. It is quite possible that strong reactions may be observed with intradermal induction concentrations lower than 1%.

CLP guidance states that "*SCLs shall be set when there is adequate and reliable scientific information available showing that the specific hazard is evident below the GCL for classification*". Thus, the setting of SCL is based on potency. However, for cyflumetofen there is no data where intradermal induction is performed with concentrations of active substance below 1% (w/w). The criteria for an extreme sensitiser (≥ 60% of animals responding following an intradermal induction of ≤ 0.1% (w/w) active substance), is not

met and consequently there is no requirement to set an SCL for sensitisation.

RAC agrees with the DS and concludes that **classification of cyflumetofen with <u>Skin Sens. 1A; H317</u> is required**.

4.6.2 Respiratory sensitisation

No specific data available.

4.6.2.1 Non-human information

None

4.6.2.2 Human information

None

4.6.2.3 Summary and discussion of respiratory sensitisation

No data available

4.6.2.4 Comparison with criteria

Not possible as no data are available.

4.6.2.5 Conclusions on classification and labelling

No classification is needed.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

The DS indicated that no specific data were available to address this endpoint and hence a comparison with the classification criteria was not possible.

Comments received during public consultation

Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal to not classify cyflumetofen for respiratory sensitisation.

Assessment and comparison with the classification criteria

As there are no data suggesting that cyflumetofen may cause respiratory sensitisation,

---

RAC proposes **no classification for respiratory sensitisation**.

4.7 Repeated dose toxicity

Table 17 Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
14 day oral (dietary) toxicity study OECD 407, rat, Fischer (F344/DuCrj) 0, 100 or 10000 mg/kg food, equal to 0, 101 and 981 mg/kg bw/d for males and 0, 105 and 1000 mg/kg bw/d for females	NOAEL: < 101 mg/kg bw/day males; <105 mg/kg bw/day females LOAEL: 101 mg/kg bw/day males; 105 mg/kg bw/day females	Increased liver and adrenal weights, vacuolation of adrenal cortical cells and vacuolisation of interstitial gland cells and/or of corpora lutea in ovary	[a]Sakai, 2001
26-day dietary non-GLP rangefinder in rat, Fischer (F344/DuCrj) 0, 500, 1500, 4000 or 12000 mg/kg food, equal to 0, 43, 128, 339, 1028 mg/kg bw/d for males and 0, 46, 132, 351 and 1039 mg/kg bw/d for females	NOAEL: 43 mg/kg bw/d males; 46 mg/kg bw/d females LOAEL: 128 mg/kg bw/day males; 132 mg/kg bw/day females	Vacuolation of adrenal cortical cells	[b]Buesen, R., 2010
28 day oral (dietary) toxicity study OECD 407, rat, Fischer (F344/DuCrj) 0, 100, 500, 1000 or 5000 mg/kg food, equal to 0, 7.50, 37.6, 75.1 and 284 mg/kg bw/day for males, and 0, 8.05, 40.8, 79.8 and 409 mg/kg bw/d for females	NOAEL: 37.6 mg/kg bw/d males; 40.8 mg/kg bw/d females LOAEL: 75.1 mg/kg bw/day males; 79.8 mg/kg bw/day females	Decreased total cholesterol and triglycerides, increased liver and adrenal weights, hepatocellular hypertrophy, vacuolation and hypertrophy adrenal cortical cells, vacuolation interstitial cells in ovary	[a]Yoshida, 2004a
28 day oral (dietary) toxicity study OECD 407, mouse, ICR (Crj :CD-1) 0, 100, 500, 1000 or 5000 mg/kg food, equal to 0, 13.1, 67.2, 135 and 663 mg/kg bw/d for males and 0, 14.5, 74.9, 150 and 763 mg/kg bw/d for females	NOAEL: 135 mg/kg bw/d males; 150 mg/kg bw/d females LOAEL: 663 mg/kg bw/day males; 763 mg/kg bw/day females	increased blood urea nitrogen, increased adrenal weight, vacuolation and hypertrophy of adrenal cortical cells, hyperplasia of adrenal subcapsular cells	[a]Yoshida, 2004b
28 day oral (gelatin capsule) toxicity study, dog, beagle (no specific OECD guideline) 0, 100, 300 or 1000 mg/kg bw/d	NOAEL: 100 mg/kg bw/d LOAEL; 300 mg/kg bw/day	Vacuolation of adrenal cortical cells, increased adrenal weight	[a]Nagashima, 2003a

Method	Results	Remarks	Reference
13 week oral (dietary) toxicity study OECD 408, rat, Fischer (F344/DuCrj) 0, 100, 300, 1000, 3000 mg/kg food, equal to 0, 5.40, 16.5, 54.5 and 167 mg/kg bw/d for males and 0, 6.28, 19.0, 62.8 and 193 mg/kg bw/d for females	NOAEL: 16.5 mg/kg bw/d males; 19.0 mg/kg bw/d females LOAEL: 54.5 mg/kg bw/day males; 62.8 mg/kg bw/day females	vacuolation for males and hypertrophy for females of the adrenal cortex, supported by decreased globulin and increased A/G ratio	[a]Yoshida, 2004c
13 week oral (dietary) toxicity study OECD 408, mouse, ICR (Crj:CD-1) 0, 300, 1000, 3000 or 10000 mg/kg food, eual to 0, 35.4, 117, 348 and 1200 mg/kg bw/d for males and 0, 45.0, 150, 447 and 1509 mg/kg bw/d for females	NOAEL: 117 mg/kg bw/d males; 150 mg/kg bw/d females LOAEL: 348 mg/kg bw/day; 447 mg/kg bw/day for females	vacuolation in females and hypertrophy in males of the adrenal cortex	[a]Yoshida, 2004d
13 week oral (gelatin capsule) toxicity study OECD 409, dog, Beagle 0, 30, 300 or 1000 mg/kg bw/d	NOAEL: 300 mg/kg bw/d LOAEL: 1000 mg/kg bw/day	reduced body weight gain, increased adrenal and testis weight and vacuolation of the adrenal cortex	[a]Nagashima, 2003b
12 months oral (gelatin capsule) toxicity study OECD 452, dog, Beagle 0, 30, 300 or 1000 mg/kg bw/d	LOAEL: 30 mg/kg bw/d	adrenal findings (mild vacuoles);	[b]Nagashima, 2004 and 2008
Long term (12 months) toxicity study in rats, Fischer (F344/DuCrj) OECD 452 0, 50, 150, 500 or 1500 mg/kg food, equal to 0, 1.9, 5.6, 18.8 and 56.8 mg/kg bw/d for males and 0, 2.3, 6.9, 23.3 and 69.2 mg/kg bw/d	NOAEL: 18.8 mg/kg bw/day in males; 23.2 mg/kg bw/day females LOAEL: 56.8 mg/kg bw/day males; 69.2 mg/kg bw/day females	Vacuolation in males and females and hypertrophy of the adrenal cortex; vacuolation of interstitial gland cells in ovaries	[a]Yoshida, 2004e
Long term (1 year) oral toxicity in rats, Fischer (F344/DuCrj) (MTD) OECD 452 0 or 6000 mg/kg food, equal to 0 or 250 mg/kg bw/d in males and 319 mg/kg bw/d in females	LOAEL: 250 mg/kg bw/day (m); LOAEL: 319 mg/kg bw/day (f)	effects on the adrenal, ovary, pancreas (males) and testis (hyperplasia of interstitial cell)	Yoshida, 2012
28 day dermal repeated dose toxicity study in Wistar rats OECD 410 0, 100, 300 or 1000 mg/kg bw/d	NOAEL >1000 mg/kg bw/day LOAEL: -	No adverse effects	[c]Buesen R. et al. 2010a

[a] As summarised in revised DAR_2011_vol3 B6 (September 2011)
[b] As summarised in addendum DAR_2011_vol3 B6 (October 2011) and agreed in PRAPeR meeting (EFSA, 2011)
[c] As summarised in OECD AII M5.3

4.7.1   Non-human information

4.7.1.1 Repeated dose toxicity: oral

14-day oral toxicity study (Sakai, 2001)

reference	:	Sakai, S., 2001	exposure	:	14 days, diet
type of study	:	14-day oral toxicity study	doses	:	0, 1000 or 10000 mg/kg food[1]
year of execution	:	2001	vehicle	:	None
test substance	:	OK-5101 (batch HN0101004; purity 98.0%)	GLP statement	:	No
route	:	Oral	guideline	:	According to OECD 407 (main study)
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Supplementary
group size	:	6/sex/dose	**NOAEL**	:	< 101 mg/kg bw/d males < 105 mg/kg bw/d females

[1] equal to 0, 101 and 981 mg/kg bw/d for males and 0, 105 and 1000 mg/kg bw/d for females

Exposure of rats to cyflumetofen (purity 98.0%) at concentrations of 0, 1000 or 10000 mg/kg food (equal to 0, 101 and 981 mg/kg bw/d for males and 0, 105 and 1000 mg/kg bw/d for females) during 14 days resulted in adverse effects in both dose groups. The study is a range finding study, performed in accordance with OECD 407 (1995). Histopathological examination of thymus, adrenals, spleen, bone with bone marrow, heart, stomach, liver, lung, kidneys, testes and ovaries was included. The results are presented in
Table 18.

Table 18 Summary table (Sakai, 2001)

Dose (mg/kg food)	0		1000		10000		dr
	m	**f**	**m**	**f**	**m**	**f**	
Mortality	No mortality						
Clinical signs -solid perineal region	0/6	0/6	0/6	0/6	0/6	6/6	
Body weight						dc	
Food consumption						dc	
Clin. Chemistry - ALP			dc		dc		m
- GOT			dc		dc		m
- GTP			dc		dc		
-creat. phosphokinase			dc		dc	dc	m
- creatinine						dc	
- total protein					ic		
- albumin					ic	ic	
- globulin						dc	
- A/G ratio			ic		ic	ic	m
- total cholesterol					dc		
- triglycerides				dc	dc	d	
- total bilirubin				dc	dc	d	

Dose (mg/kg food)	0		1000		10000		dr
	m	f	m	f	m	f	
Urinalysis	No treatment-related changes						
Organ weights - thymus - liver - spleen - kidneys - adrenals - testis	 ic^r		 ic^r ic^r	 $ic^{a,r}$	$dc^{a,r}$ $ic^{a,r}$ ic^r $ic^{a,r}$ ic^r	 $ic^{a,r}$ dc^a ic^r $ic^{a,r}$	 m m,f
Pathology <u>macroscopy</u> -adrenal, hypertrophy	0/6	0/6	0/6	5/6	6/6	6/6	m,f
<u>microscopy</u> **liver:** -hypertrophy, hepatocyte, diffuse	0/6	0/6	0/6	0/6	6/6	6/6	
adrenal: -vacuolation, cortical cell, diffuse	0/6	0/6	6/6	6/6	6/6	6/6	
ovary: -vacuolation, interstitial cell		0/6		6/6		6/6	
-vacuolation, corpora lutea		0/6		0/6		6/6	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative

Table 19 Mean body weight of rats administered Cyflumetofen for 14 days

	Males			Females		
Dose level [ppm]	0	1000	1000	0	1000	1000
Body weight [g]						
- Day 0	88.7	86.3	91.4	75.9	77.2	73.4
- Day 14	152.1	144.8	145.8	113.9	112.8	102.4*
Overall body weight gain [g]	63.4	58.5	54.5	38.0	35.6	29.0
Gain (Percent of Control)	100	92.3	86.0	100	93.7	76.3
* or **: Significantly different from control value, p<0.05 or P<0.01, respectively						

In females at 10000 mg/kg food, soiled perineal region, decreased body weight and food consumption were noted. Clinical chemistry showed in males at 1000 and 10000 mg/kg food decrease in alkaline phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and creatine phosphokinase and an increase in albumin/globulin ratio. Males at 10000 mg/kg food showed also an increase in total protein and albumin and a decrease in total cholesterol, triglyceride and total bilirubin. Females at 1000 mg/kg food showed a decrease in creatine phosphokinase, creatine and globulin and an increase in albumin and albumin/globulin ratio. In females at 1000 mg/kg food a decrease in triglyceride and total bilirubin were noted.

A decrease in absolute and relative thymus weight was noted in males (80 and 84% of controls, respectively) at 10000 mg/kg food. In males, absolute liver weight was increased in males at 10000 mg/kg food (137% of controls) and relative liver weight was increased at 1000 and 10000 mg/kg food (110 and 144% of controls). In females, absolute and relative liver weight was increased at 10000 mg/kg food (119 and 131% of controls). In females at 10000 mg/kg food, relative spleen weight was decreased (88% of controls). Relative kidney weights were increased in males and females at 10000 mg/kg food (108 and 109-111% of controls). Absolute adrenal weights were increased in males at 10000 mg/kg food (165-195% of controls (left-right)), and in females at 1000 and 10000 mg/kg food (133-139 and 172-161% of controls). Relative adrenal weight were increased males at 1000 and 10000 mg/kg food (115-136 and 162-200% of controls) and in females at 1000 and 10000 mg/kg food (133-139 and 178-183% of controls). Relative testis weights were increased in males at 10000 mg/kg food (116% of controls).

At necropsy, diffuse hyperthrophy of the hepatocytes in the liver was observed in all males and females at 10000 mg/kg food. In all males and females at 1000 and 10000 mg/kg food, diffuse vacuolation of cortical cells in adrenals was observed. All females at 1000 and 10000 mg/kg food showed vacuolation of interstitial gland cells in ovary and/or vacuolation of corpora lutea in the ovary.

Based on the effects noted in liver, adrenals and ovary, the NOAEL is set at < 1000 mg/kg food (equal to <101 and <105 mg/kg bw/d for males and females, respectively).

28-day oral toxicity studies

26-day oral toxicity study in rats (Buesen, 2010)

reference	:	Buesen 2010	exposure	:	26 days, diet
type of study	:	26-day oral toxicity study	doses	:	0, 500, 1500, 4000 or 12000 mg/kg food[1]
year of execution	:	2010	vehicle	:	None
test substance	:	OK-5101 purity not specified	GLP statement	:	No
route	:	Oral	guideline	:	-
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Supplementary
group size	:	5/sex/dose	**NOAEL**	:	43 mg/kg bw/d males 46 mg/kg bw/d females

[1] equal to 0, 43, 128, 339 and 1028 mg/kg bw/d for males and 0, 46, 132, 351 and 1039 mg/kg bw/d for females

Exposure of rats in a non-GLP range-finding study to cyflumetofen (purity not reported) for 26 days at concentrations of 0, 500, 1500, 4000 or 12000 mg/kg food (equal to 0, 43, 128, 339 and 1028 mg/kg bw/d for males and 0, 46, 132, 351 and 1039 mg/kg bw/d for females) resulted in effect in the three highest dose groups. The results are presented in Table 20, Table 21 and Table 22. In the high dose group, decreased body weight (gain) and decreased food consumption was observed, together with centrilobular, hepatocellular hypertrophy in the liver, adrenocortical, diffuse macrovesicular vacuolation and macrovesicular vacuolation of luteal cells of corpora lutea in the ovary. At 4000 ppm decreased body weight gain was observed in males, and centrilobular, hepatocellular hypertrophy in the liver of males and in one female, adrenocortical multifocal macrovesicular vacuolation and foci of macrovesicular vacuolation of luteal cells of corpora lutea in the ovary. At 1500 ppm adrenocortical, single foci of macrovesicular vacuolation in some males and females was observed. Based on the adrenal effects (cortical cell vacuolation), the NOAEL is set at 500 ppm (equal to 43 and 46 mg/kg bw/d for males and females, respectively).

Table 20 Mean body weight of rats administered cyflumetofen for 26 days (Buesen, 2010)

	MALE					FEMALE				
Dose level [ppm]	0	500	1500	4000	12000	0	500	1500	4000	12000
Body weight [g]										
- Day 0	187.78	188.36	187.86	187.84	185.52	139.96	140.42	137.48	140.10	138.54
- Day 26	314.32	311.52	302.22	298.80	286.08*	191.10	191.48	188.30	181.96	172.32*
Overall body weight gain [g]	126.54	123.16	114.36	110.96	100.56**	51.14	51.06	50.82	41.86	33.78 **
Change (% of Control)	100	97.33	90.37	87.69	79.47	100	99.84	99.37	81.85	66.05

* or **: Significantly different from control value, $p<0.05$ or $p<0.01$, respectively

Table 21 Selected mean absolute and relative organ weights of rats administered cyflumetofen for 26 days) (Buesen, 2010)

Sex		MALE				FEMALE			
		Absolute Weight		Relative Weight		Absolute Weight		Relative Weight	
Organ weight	Dose [ppm]	[mg] or [g]	% of Control	[% of b.w.]	% of Control	[mg] or [g]	% of Control	[% of b.w.]	% of Control
Adrenal (mg)	0	64.2	100	0.022	100	72.2	100	0.041	100
	500	62.8	98	0.021	98	94.8*	131	**0.053****	130
	1500	67.0	104	0.024	108	**96.6****	134	**0.056****	136
	4000	**87.8 ****	137	**0.032****	145	**132.4****	183	**0.078****	192
	12000	**108.2****	169	**0.041****	187	**131.2****	182	**0.082****	201
Liver (g)	0	7.558	100	2.551	100	4.636	100	2.638	100
	500	7.652	101	2.602	102	4.768	103	2.684	102
	1500	7.548	100	2.657	104	4.82	104	2.767	105
	4000	**8.6 ***	114	**3.092****	121	4.936	106	**2.927****	111
	12000	**8.782***	116	**3.301****	129	**5.52****	119	**3.462****	131
Ovary (mg)	0	-	-			93.8	100	0.054	100
	500	-	-			100.0	107	0.056	105
	1500	-	-			95.6	102	0.055	102
	4000	-	-			**120.8****	129	**0.072****	134
	12000	-	-			105.6	113	0.066	123

* or **: Significantly different from control value, $p<0.05$ or $p<0.01$, respectively

Table 22 Selected histopathological lesions (Incidence) in rats administered cyflumetofen for 26 day (Buesen, 2010)

Organ / Lesion		MALE					FEMALE				
Dose (ppm)		0	500	1500	4000	12000	0	500	1500	4000	12000
Liver	Infiltration, lymphoid	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5	5/5
	Hypertrophy, centrilobular	0/5	0/5	0/5	5/5	5/5	0/5	0/5		1/5	5/5
Adrenal	Adrenal cortex cytoplasmic vacuolation	0/5	0/5	3/5	5/5	5/5	0/5	0/5	3/5	5/5	5/5
Ovary	Vacuolation, luteal cells						0/5	0/5	0/5	4/5	5/5

28-day oral toxicity study in rats (Yoshida, 2004a)

reference	:	Yoshida, T., 2004a	exposure	:	28 days, diet
type of study	:	28-day oral toxicity study	doses	:	0, 100, 500, 1000 or 5000 mg/kg[1] food
year of execution	:	2001	vehicle	:	None
test substance	:	OK-5101 (batch 01C1; purity 99.3%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 407 (main study)
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Acceptable
group size	:	6/sex/dose	**NOAEL**	:	37.6 mg/kg bw/d (M) 40.8 mg/kg bw/d (F)

[1] equal to 0, 7.50, 37.6, 75.1 and 384 mg/kg bw/d for males and 0, 8.05, 40.8, 79.8 and 409 mg/kg bw/d for females

Exposure of rats to cyflumetofen (purity 99.3%) at concentrations of 0, 100, 500, 1000 or 5000 mg/kg food (equal to 0, 7.50, 37.6, 75.1 and 384 mg/kg bw/d for males and 0, 8.05, 40.8, 79.8 and 409 mg/kg bw/d for females) during 28 days resulted in several effects in the two highest dose groups. A summary of the results is presented in
Table 23. In Table 24 and Table 25 detailed results for clinical biochemistry and organ weights are provided.

Table 23 Summary table (Yoshida, 2004a)

Dose (mg/kg food)		0		100		500		1000		5000		dr
		m	f	m	f	m	f	m	f	m	f	
Mortality						none						
Clinical signs						no treatment-related findings						
Body weight						no treatment-related findings						
Food consumption						no treatment-related findings						
Haematology - WBC (%)							i; 115		i; 110		i; 110	

Dose (mg/kg food)	0		100		500		1000		5000		dr
	m	f	m	f	m	f	m	f	m	f	
Clin. Chemistry											
- ALP								dc	dc		
- BUN									ic		
- total cholesterol							dc		dc	dc	
- triglycerides							dc		dc	d	m
- potassium							ic		ic		
- sodium										dc	
- creatine kinase				i		i		i		d	
Urinalysis											
- ketones										ic	
Organ weights											
- liver							$ic^{a,r}$		$ic^{a,r}$	$ic^{a,r}$	m
- kidneys									$ic^{a,r}$	$ic^{a,r}$	
- adrenals								i^a, ic^r	$ic^{a,r}$	$ic^{a,r}$	f
- ovary										i	
Pathology											
macroscopy											
-adrenal, hypertrophy and white in colour	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	6/6	6/6	
microscopy											
liver:											
-hypertrophy, hepatocyte, diffuse	0/6	0/6	0/6	0/6	0/6	0/6	6/6	0/6	6/6	6/6	
adrenal:											
-vacuolation, cortical cell, diffuse	0/6	0/6	0/6	0/6	0/6	0/6	6/6	6/6	6/6	6/6	
-hypertrophy, cortical cell, diffuse	0/6	0/6	0/6	0/6	0/6	0/6	0/6	6/6	0/6	6/6	
ovary:											
-vacuolation, interstitial cell		0/6		0/6		0/6		2/6		6/6	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative

Table 24 Selected clinical chemistry findings in rats administered cyflumetofen for 28 days (group means) (Yoshida, 2004a)

Parameter	Group mean (% of the control value)							
	MALE				FEMALE			
	100	500	1000	5000	100	500	1000	5000
Alkaline phosphatase (ALP)	101	95	94	87**	95	96	88**	94

Parameter	Group mean (% of the control value)							
	MALE				FEMALE			
	100	500	1000	5000	100	500	1000	5000
Blood urea nitrogen (BUN)	101	103	99	115**	95	94	100	104
Total cholesterol (T.Chol)	102	98	93*	93*	100	100	100	88*
Triglyceride (TG)	96	95	80**	68*	114	114	102	88
Sodium (Na)	100	100	100	100	100	100	98	95**
Potassium (K)	99	101	99	107*	97	99	99	96

* p ≤ 0.05; ** p ≤ 0.01

Table 25 Selected mean absolute and relative organ weights of rats administered cyflumetofen for 28 days (Yoshida, 2004a)

Sex		MALE				FEMALE			
Organ weight	Dose [ppm]	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control
Pituitary [mg]	0	7.1	100	0.0034	100	8.4	100	0.0065	100
	100	7.8	110	0.0036	106	8.5	101	0.0067	103
	500	7.9	111	0.0038	112	9.0	107	0.0070	108
	1000	7.9	111	0.0036	106	8.0	95	0.0062	95
	5000	7.8	110	0.0037	109	9.8	117	0.0075	115
Liver [g]	0	6.14	100	2.92	100	3.52	100	2.71	100
	100	6.49	106	2.97	102	3.46	98	2.71	100
	500	6.38	104	3.04	104	3.50	99	2.72	100
	1000	**6.76***	**110***	**3.14****	**108****	3.50	99	2.73	101
	5000	**8.03****	**131****	**3.76****	**129****	**4.07****	**116****	**3.14****	**116****
Kidneys [mg]	0	1455	100	0.69	100	961	100	0.74	100
	100	1553	107	0.71	103	965	100	0.75	101
	500	1504	103	0.72	104	978	102	0.76	103
	1000	1535	105	0.71	103	986	103	0.77	104
	5000	**1658****	**114****	**0.78****	**113****	**1035***	**108***	**0.80****	**108****
Adrenals [mg]	0	36.9	100	0.018	100	43	100	0.033	100
	100	36.9	100	0.017	94	45.6	106	0.036	109

Sex		MALE				FEMALE			
Organ weight	Dose [ppm]	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control
	500	37.7	102	0.018	100	46.4	108	0.036	109
	1000	38.7	105	0.018	100	48.8	113	**0.038***	**115***
	5000	**50.3****	**136****	**0.024****	**133****	**85.8****	**200****	**0.066****	**200****

* p ≤ 0.05; ** p ≤ 0.01

Clinical signs of toxicity observed throughout the study were not dose related. No mortality was observed in this study. Body weight development and food consumption were not affected by treatment.

There were no statistically significant changes in haematological parameters; in females increases in white blood cells counts were noted at 500, 1000 and 5000 mg/kg food (115, 110 and 110%, respectively), but were not considered toxicological relevant since changes were not dose related. Clinical chemistry showed a decreased alkaline phosphatase at 1000 mg/kg food in females (88% of controls) and 5000 mg/kg food in males (87% of controls); a decrease is not considered to be toxicologically relevant. Blood urea nitrogen was increased at 5000 mg/kg food in males (115% of control). Total cholesterol and triglycerides were decreased at 1000 mg/kg food in males (93 and 80% of control, respectively) and at 5000 mg/kg food in males (93 and 68% of control, respectively) and females (88% of control for both). Potassium was increased at 5000 mg/kg food in males (107% of control) and sodium was decreased at 5000 mg/kg food in females (95% of control). Non-statistically significant changes in creatine phosphokinase were observed in females in all doses levels (291, 148, 125 and 92% of controls); however, these changes were not considered toxicologically relevant since changes were not dose related.

At urinalysis an increased amount of ketones was noted at 5000 mg/kg food in females.

Absolute and relative liver weight was increased at 1000 mg/kg food in males (110 and 108% of control, respectively), at 5000 mg/kg food in males (131 and 129% of control, respectively) and females (116% of control for both). Absolute and relative kidney weight was increased at 5000 mg/kg food in males (114 and 113% of control) and females (108% of control for both). Absolute and relative adrenal weight was increased at 1000 mg/kg food in females (113 and 115% of control), and at 5000 mg/kg food in males (136 and 133% of control) and females (200% of control for both). A non-statistically significant increase in absolute and relative ovary weight was noted in females at 5000 mg/kg food (111% of control for both).

At necropsy, hyperthrophy and adrenals that were white in colour were observed in males and females at 5000 mg/kg food. Histopathological examination revealed diffuse vacuolation of cortical cells in the adrenals in both sexes at 1000 and 5000 mg/kg food. Diffuse hypertrophy of hepatocytes in the liver was noted in males at 1000 mg/kg food and both sexes at 5000 mg/kg food. Females at 1000 and 5000 mg/kg food showed also vacuolation of the cortical cells in the adrenals. Vacuolation of interstitial cells in the ovaries was noted in females at 5000 mg/kg food.

The results of lipid staining performed on the adrenal of both sexes and ovaries of females indicated that the vacuolation observed in these organs was lipid deposition.

Based on decreased total cholesterol and triglycerides, increased liver and adrenal weights, and histopathological changes in the liver, adrenals and ovaries, the NOAEL is set at 500 mg/kg food (equal to 37.6 and 40.8 mg/kg bw/d for males and females, respectively).

28-day oral toxicity study in mice (Yoshida, 2004b)

reference	:	Yoshida, T., 2004b	exposure	:	28 days, diet
type of study	:	28-day oral toxicity study	doses	:	0, 100, 500, 1000 or 5000 mg/kg[1] food
year of execution	:	2004	vehicle	:	None
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 407
species	:	Mouse, ICR (Crj :CD-1)	acceptability	:	Acceptable
group size	:	6/sex/dose	**NOAEL**	:	135 mg/kg bw/d (M) 150 mg/kg bw/d (F)

[1] equal to 0, 13.1, 67.2, 135 and 663 mg/kg bw/d for males and 0, 14.5, 74.9, 150 and 763 mg/kg bw/d for females

Exposure of mice to cyflumetofen (purity 97.67%) at concentrations of 0, 100, 500, 1000 or 5000 mg/kg food (equal to 0, 13.1, 67.2, 135 and 663 mg/kg bw/d for males and 0, 14.5, 74.9, 150 and 763 mg/kg bw/d for females) during 28 days resulted in effects on blood urea nitrogen concentration and on adrenals in high dose animals. The results are presented in Table 26 and Table 27.

Table 26 Summary table (Yoshida, 2004b)

Dose (mg/kg food)	0		100		500		1000		5000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	none										
Clinical signs	no treatment-related findings										
Body weight	no treatment-related findings										
Food consumption	no treatment-related findings										
Haematology - platelet count (%)	100	87	94	99	97	93	94	86*			
Clin. Chemistry - BUN										i	
Organ weights - thyroids - lungs - spleen - adrenals			d[a,r]	dc[a,r]	d[a,r]		d[a,r] dc[a], d[r]		d[a,r] i[a,r]	dc[a,r] i[a,r]	
Pathology											
macroscopy	no treatment-related findings										

Dose (mg/kg food)	0		100		500		1000		5000		dr
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
microscopy adrenal: -vacuolation, cortical cell, diffuse	1/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6	1/6	5/6	
-hypertrophy, cortical cell, diffuse	0/6	1/6	0/6	1/6	0/6	0/6	0/6	1/6	4/6	6/6	
-hyperplasia, subcapsular cell	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative
* $p \leq 0.05$; ** $p \leq 0.01$

Table 27 Selected mean absolute and relative organ weights of mice administered cyflumetofen for 28 days (Yoshida, 2004b)

Sex		MALE				FEMALE			
Organ weight	Dose [ppm]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
Pituitary	0	2.3	100	0.0060	100	2.7	100	0.0086	100
	100	2.3	100	0.0056	93	2.1	78	0.0068	79
	500	2.4	104	0.0060	100	2.5	93	0.0078	91
	1000	2.0	87	0.0050	83	2.6	96	0.0078	91
	5000	2.2	96	0.0055	92	2.2	81	0.0069	80
Lung	0	198	100	0.52	100	196	100	0.63	100
	100	207	105	0.50	96	167*	85*	0.54**	86**
	500	208	105	0.53	102	200	102	0.62	98
	1000	204	103	0.52	100	186	95	0.57	90
	5000	208	105	0.52	100	164*	84*	0.52**	83**
Spleen	0	124	100	0.32	100	152	100	0.49	100
	100	128	103	0.31	97	137	90	0.43	88
	500	123	99	0.31	97	142	93	0.44	90
	1000	103*	83*	0.26	81	149	98	0.46	94
	5000	116	94	0.29	91	138	91	0.44	90
Adrenals	0	5.0	100	0.013	100	9.7	100	0.031	100
	100	4.6	92	0.011	85	8.6	89	0.028	90
	500	4.9	98	0.012	92	9.9	102	0.031	100
	1000	5.2	104	0.013	100	9.9	102	0.031	100
	5000	6.2	124	0.015	115	11.7	121	0.037	119
Thyroid	0	4.9	100	0.013	100	3.7	100	0.012	100

Sex		MALE				FEMALE			
Organ weight	Dose [ppm]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
	100	4.3	88	0.010	77	3.9	105	0.013	108
	500	4.3	88	0.011	85	3.7	100	0.012	100
	1000	4.1	84	0.011	85	4.0	108	0.012	100
	5000	3.9	80	0.010	77	3.8	103	0.012	100

* p ≤ 0.05; ** p ≤ 0.01

No mortality was observed in this study. Clinical signs, body weight development and food consumption were not affected by treatment.

The statistically significant decrease in platelet count in females at 5000 mg/kg food was not considered treatment related, since the platelet count at 100 mg/kg food was also decreased with almost the same degree and therefore no dose-relationship was apparent.

Clinical chemistry showed increased blood urea nitrogen at 5000 mg/kg food in females (114% of control). Further changes in clinical chemistry were not considered treatment related since there was no dose-relationship.

Absolute and relative thyroid weight was non-statistically significantly decreased at all dose levels in males (80-88% and 77-85% of control, respectively), without a dose-relationship for the relative weight. Absolute and relative lung weight was decreased at 100 and 5000 mg/kg food in females (84-85% and 83-86% of control, respectively). Absolute and relative spleen weight was decreased at 1000 mg/kg food in males (83 and 81% of control, respectively). As no dose-relationship was present, changes were not accompanied by necropsy findings, biochemical changes and/or haematological changes, the above decreases in thyroid, lung and spleen weight were considered to be not treatment-related. Absolute and relative adrenal weight was increased at 5000 mg/kg food in males (124 and 115% of control) and females (121 and 119% of control).

At macroscopy no abnormalities were detected. Histopathology revealed diffuse vacuolation of adrenal cortical cells in one male at 0, 100 and 5000 mg/kg food and 5 females at 5000 mg/kg food. Diffuse hypertrophy of adrenal cortical cells was noted in 4 males at 5000 mg/kg food and one female at 0, 100 and 1000 mg/kg food and all females at 5000 mg/kg food. One female at 5000 mg/kg food showed also subcapsular cell hyperplasia. The severity of diffuse vacuolation (slight) and hypertrophy (slight) seen in one animal at 100 and/or 1000 mg/kg food was comparable to that seen in the control group and was therefore not considered to be treatment-related.

Based on increased blood urea nitrogen, increased adrenal weights, and histopathology in the adrenals, the NOAEL is set at 1000 mg/kg food (equal to 135 and 150 mg/kg bw/d for males and females, respectively).

28-day oral toxicity study in dogs (Nagashima, 2003a)

reference	:	Nagashima, Y., 2003a	exposure	:	28 days, gelatin capsule
type of study	:	28-day oral toxicity study	doses	:	: 0, 100, 300 or 1000 mg/kg bw/d
year of execution	:	2001	vehicle	:	None
test substance	:	OK-5101 (batch 01H1; purity 98.4%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD Guidelines for testing of chemicals (no specific

				OECD guideline available for a range-finding study in dogs)
species	:	Dog, beagle	acceptability	: Acceptable
group size	:	3/sex/dose	**NOAEL**	: 100 mg/kg bw/d

Dogs were given 0, 100, 300 or 1000 mg/kg bw/d cyflumetofen (purity 98.4%) during 28 days by gelatine capsule. The results are presented in Table 28 and Table 29.

Table 28 Summary table (Nagashima, 2003a)

Dose (mg/kg bw)	0		100		300		1000		dr
	m	f	m	f	m	f	m	f	
Mortality	no treatment-related findings								
Clinical signs (n=3) - faeces with test substance					+	+	++	++	m/f
Body weight	no treatment-related findings								
Food consumption	no treatment-related findings								
Ophthalmoscopy	no treatment-related findings								
Haematology	no treatment-related findings								
Clin. Chemistry - potassium (mmol/L) - calcium (mg/dL) - total bilirubin (mg/dL)	4.0 10.3 0.10	4.3 10.8 0.09	4.4 10.9 0.06	4.3 10.8 0.09	4.7* 11.0* 0.05*	4.3 10.8 0.09	4.6* 11.0* 0.07	4.6 10.7 0.09	
Urinalysis	no treatment-related findings								
Organ weights - adrenals					i[a/r]	i[a/r]	i[a/r]	i[a/r]	
Pathology									
macroscopy -heart, right atrioventiricular valve, discoloured red foci	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3	
microscopy adrenal: -fine vacuoles, cortical cell, zona fasciculata and reticularis	0/3	0/3	0/3	0/3	2/3	3/3	3/3	3/3	m/f
-fine vacuoles, cortical cell, zona glomerulosa	0/3	0/3	0/3	0/3	1/3	0/3	2/3	2/3	m/f

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
+   present in part of the animals
++   present in all animals

* p ≤ 0.05; ** p ≤ 0.01

Table 29 Selected mean absolute and relative organ weights of dogs administered cyflumetofen for 28 days (Nagashima, 2003a)

Sex		MALE				FEMALE			
Organ weight	Dose [mg/kg bw]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
Adrenal (Right)	0	439	100	5.07	100	446	100	5.75	100
	100	478	109	5.28	104	388	87	5.00	87
	300	501	114	5.54	109	562	126	7.06	123
	1000	572	130	6.54	129	579	130	7.95	138
Adrenal (Left)	0	437	100	5.03	100	445	100	5.73	100
	100	470	108	5.19	103	422	95	5.41	94
	300	512	117	5.68	113	574	129	7.21	125
	1000	534	124	6.20	123	510	115	7.00	122

Mortality, clinical signs, body weight development and food consumption were not affected by treatment.

Clinical observations revealed faeces with test substance –like white substance in all animals at 1000 mg/kg bw/d during the study period and less frequent in animals at 300 mg/kg bw/d. Body weights, food consumption, ophthalmoscopy, haematology, clinical biochemistry and urinalysis showed no treatment-related findings. The increased potassium and calcium relative to the control group in males at 300 and 1000 mg/kg bw/d was considered to be toxicologically insignificant as increases were slight, the value in the control group was low compared to pre-test values and/or the values were within background data. Decreased total bilirubin at 300 mg/kg bw/d in males was considered not toxicologically relevant since the value in the control group was high compared to pre-test values and there was no dose-response relationship. Increases in absolute and relative adrenal weights were noted in 1 male and 2 females at 300 mg/kg bw/d and in 2 males and 2 females at 1000 mg/kg bw/d.

Macroscopy showed dark red foci on the right atrioventricular valve of the heart, which could be explained by capillary dilation at histopathology. This is a normal finding sometimes seen in beagle dogs. Histopathology revealed fine vacuoles in adrenal cortical cells in the zona fasciculata and reticularis in 2 males and 3 females at 300 mg/kg bw/d and in all animals at 1000 mg/kg bw/d. Fine vacuoles were also seen in the zona glomerulosa in one male at 300 mg/kg bw/d and 2 animals of each sex at 1000 mg/kg bw/d.

Based on vacuolation of adrenal cortical cells and the increase in adrenal weight at 300 mg/kg bw/d, the NOAEL is set at 100 mg/kg bw/d.

90-day and 1-year oral toxicity studies

13-week oral toxicity study in rats (Yoshida, 2004c)

reference	:	Yoshida, T., 2004c	exposure	:	13 weeks, diet
type of study	:	13-week oral toxicity study	doses	:	: 0, 100, 300, 1000, 3000 mg/kg food[1]
year of execution	:	2001	vehicle	:	None
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 408
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	16.5 mg/kg bw/d (M) 19.0 mg/kg bw/d (F)

[1] equal to 0, 5.40, 16.5, 54.5 and 167 mg/kg bw/d for males and 0, 6.28, 19.0, 62.8 and 193 mg/kg bw/d for females

Rats were given cyflumetofen (purity 97.67%) at dose levels of 0, 100, 300, 1000 or 3000 mg/kg food (equal to 0, 5.40, 16.5, 54.5 and 167 mg/kg bw/d for males and 0, 6.28, 19.0, 62.8 and 193 mg/kg bw/d for females) during 13 weeks and sacrificed after 13 weeks. A summary of the results is presented in Table 30. Some selected detailed results are presented in Table 31, Table 32 and Table 33.

Table 30 Summary table (Yoshida, 2004c)

Dose (mg/kg food)	0		100		300		1000		3000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality				no treatment-related findings							
Clinical signs (n=10) - rearing	9	5	6	2	3	1	2	4	0	1	
Functional observations - motor activity - grip strength forelimb					dc		ic	ic			
Body weight				no treatment-related findings							
Food consumption				no treatment-related findings							
Ophthalmoscopy				no treatment-related findings							
Haematology - prothrombin time - total leukocyte count - myeloid/erythroid			d	d	dc		dc	dc	ic	dc	
Clin. Chemistry - globulin - A/G ratio							dc ic	dc ic			
Urinalysis - ketones					dc						
Organ weights - liver - kidneys - adrenals - heart					ic[r] dc[a]		ic[r]		ic[a,r] ic[r]	ic[r] ic[r] ic[a,r] dc[a]	

Dose (mg/kg food)	0		100		300		1000		3000		dr
	m	f	m	f	m	f	m	f	m	f	
Pathology											
macroscopy											
- adrenal, enlarged and white in colour	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	
microscopy											
adrenal:											
- vacuolation, cortical cell, diffuse	0/10	0/10	0/10	0/10	0/10	0/10	6/10	0/10	10/10	0/10	m
- hypertrophy, cortical cell, diffuse	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	0/10	10/10	
ovary:											
- vacuolation, interstitial gland cell		0/10		0/10		0/10		1/10		8/10	f

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative

Table 31 Selected haematology findings in rats administered cyflumetofen for 13 weeks (Yoshida, 2004c)

Parameter	Group mean (% of the control value)							
	MALE				FEMALE			
Dose level	100	300	1000	3000	100	300	1000	3000
Prothrombin Time (PT)	105	101	101	117**	99	102	102	96
Total leukocyte count (WBC)	106	102	108	105	95	97	84**	88*
Myeloid/erythroid (M/E) ratio	-	-	-	-	90	82	70**	75

$* p \leq 0.05$; $** p \leq 0.01$

Table 32 Selected clinical chemistry findings in rats administered cyflumetofen for 13 weeks (group means) (Yoshida, 2004c)

Parameter	Group mean (% of the control value)							
	MALE				FEMALE			
Dose level	100	300	1000	3000	100	300	1000	3000
Globulin (Glob)	99	100	101	102	98	97	93**	92**
Albumin / globulin ratio	101	100	99	101	102	103	110**	112**

$* p \leq 0.05$; $** p \leq 0.01$

Table 33 Selected mean absolute and relative organ weights of rats administered cyflumetofen for 13 weeks (Yoshida, 2004c)

Sex	MALE	FEMALE

Organ	Dose [ppm]	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg] or [g]	% of Control	Relative weight [% of b.w.]	% of Control
Heart [mg]	0	921	100	0.3	100	533	100	0.32	100
	100	875	95	0.3	100	536	101	0.32	100
	300	856	93*	0.29	97	551	103	0.33	103
	1000	875	95	0.29	97	532	100	0.32	100
	3000	866	94*	0.29	97	540	101	0.33	103
Liver [g]	0	6.84	100	2.22	100	3.55	100	2.11	100
	100	6.61	97	2.22	100	3.61	102	2.16	102
	300	6.82	100	2.27	102	3.58	101	2.13	101
	1000	6.84	100	2.31	104**	3.53	99	2.15	102
	3000	7.37	108*	2.48	112**	3.63	102	2.23	106**
Kidneys [mg]	0	1846	100	0.60	100	1051	100	0.63	100
	100	1782	97	0.60	100	1061	101	0.64	102
	300	1850	100	0.62	103	1083	103	0.64	102
	1000	1870	101	0.63	105	1041	99	0.63	100
	3000	1910	103	0.64	107*	1060	101	0.65	103*
Adrenals [mg]	0	44.8	100	0.015	100	42.8	100	0.026	100
	100	40.5	90	0.014	93	43.6	102	0.026	100
	300	40.7	91	0.014	93	42.8	100	0.026	100
	1000	42.7	95	0.014	93	45.5	106	0.028	108*
	3000	47.3	106	0.016	107	60.8	142**	0.037	142**

* $p \leq 0.05$; ** $p \leq 0.01$

Clinical observations revealed a significant decrease in rearing of males in week 1 of treatment. As during the remaining study period no difference between control and treated animals was noted, this decrease is not considered to be an adverse effect. Functional observations showed an increased motor activity at one 10-min interval in males and females at 1000 mg/kg food. Grip strength of the forelimb was decreased in males at 300 mg/kg food. As no effects were seen at higher levels, the effects observed are considered to be incidental and not related to treatment.

Body weight development, food consumption and ophthalmoscopy (control and at 3000 mg/kg food) were not affected by treatment.

Prothrombin time was increased in males at 3000 mg/kg food (117% of control). Total leukocyte count was decreased in females at 1000 and 3000 mg/kg food (84 and 88% of control). Bone marrow cytology showed the myeloid/erythroid ratio to be decreased in all females (70 to 90% of control, see Table 34). In the absence of a dose-response relationship for total leukocyte count and myeloid/erythroid ratio these effects are not considered to be treatment-related.

Clinical chemistry showed that globulin was decreased (93 and 92% of control) and the A/G ratio was increased (110 and 112% of control) in females at 1000 and 3000 mg/kg food.

The reduced amount of ketones in males at 300 mg/kg food at urinalysis was not considered to be treatment-related as no effect was observed at 1000 and 3000 mg/kg food.

Absolute liver weight was slightly increased at 3000 mg/kg food in males (108% of control) and relative liver weight was slightly increased at 1000 mg/kg food in males (104% of control) and 3000 mg/kg food in males and females (112 and 106% of control, respectively). Relative kidney weight was slightly increased at 3000 mg/kg food in males and females (107 and 103% of control, respectively). Absolute and relative adrenal weight was increased at 3000 mg/kg food in females (142% of control for both). Relative adrenal weight was also increased at 1000 mg/kg food in females (108% of control). The decrease in absolute heart weight in males at 300 and 3000 mg/kg

food (93 and 94% of control, respectively) was not considered to be treatment-related as the decrease was not dose-related and minimal.

Macroscopy showed enlarged and discoloured adrenals in all females at 3000 mg/kg food. Histopathology revealed diffuse vacuolation of adrenal cortical cells in 6/10 males at 1000 mg/kg food and all males at 3000 mg/kg food. Diffuse hypertrophy of adrenal cortical cells was seen in all females at 1000 and 3000 mg/kg food, with a dose-related increase in severity. Vacuolation of interstitial gland cells in the ovaries was noted in 1/10 (not statistically significant) and 8/10 females at 1000 and 3000 mg/kg food, respectively.

Based on vacuolation for males and hypertrophy for females of the adrenal cortex at 1000 and 3000 mg/kg food, the NOAEL is set at 300 mg/kg food (equal to 16.5 and 19.0 mg/kg bw/d for males and females, respectively). The decreased globulin and increased A/G ratio, although slight effects, support the NOAEL of 300 mg/kg food.

13-week oral toxicity study in mice (Yoshida, 2004d)

reference	:	Yoshida, T., 2004d	exposure	:	13 weeks, diet
type of study	:	13-week oral toxicity study	doses	:	: 0, 300, 1000, 3000, 10000 mg/kg food[1]
year of execution	:	2001	vehicle	:	None
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 408
species	:	Mouse, ICR (Crj:CD-1)	acceptability	:	Acceptable
group size	:	10/sex/dose	**NOAEL**	:	117 mg/kg bw/d (M) 150 mg/kg bw/d (F)

[1] 0, 35.4, 117, 348 and 1200 mg/kg bw/d for males and 0, 45.0, 150, 447 and 1509 mg/kg bw/d for females

Exposure of mice to cyflumetofen (purity 97.67%) at concentrations of 0, 300, 1000, 3000 or 10000 mg/kg food (equal to 0, 35.4, 117, 348 and 1200 mg/kg bw/d for males and 0, 45.0, 150, 447 and 1509 mg/kg bw/d for females) during 13 weeks resulted in effects on the adrenal cortex of males and females dosed at and above 3000 mg/kg food. The summary of results is presented in Table 35. More detailed results are presented in Table 36 and Table 37.

Table 35 Summary table (Yoshida, 2004d)

Dose (mg/kg food)	0		300		1000		3000		10000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	no treatment-related findings										
Clinical signs	no treatment-related findings										
Body weight	no treatment-related findings										
Food consumption	no treatment-related findings										
Haematology - MCHC								ic			
Clin. Chemistry											

Dose (mg/kg food)	0		300		1000		3000		10000		dr
	m	f	m	f	m	f	m	f	m	f	
- ASAT					dc		dc		dc		
- ALAT							dc				
- BUN							dc				
Organ weights											
- adrenals									ic[a,r]	i[a,r]	
- thyroid			i		i		i		i		
Pathology											
macroscopy											
- adrenal, enlarged	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	9/10	0/10	
microscopy											
adrenal:											
-vacuolation, cortical cell, diffuse	0/10	0/10	0/10	0/10	0/10	0/10	0/10	2/10	0/10	7/10	
-hypertrophy, cortical cell, diffuse	0/10	0/10	0/10	0/10	0/10	0/10	1/10	0/10	1/10	0/10	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
a/r   absolute/relative

Table 36 Selected clinical chemistry findings in mice administered cyflumetofen for 13 weeks (group means) (Yoshida, 2004d)

Parameter	Group mean (% of the control value)							
	MALE				FEMALE			
Dose level	300	1000	3000	10000	300	1000	3000	10000
Glutamic oxaloacetic transaminase (GOT)	54	48*	47*	50*	111	107	109	102
Glutamic pyruvic transaminase (GPT)	49	47*	43*	51	100	100	92	92
Blood urea nitrogen (BUN)	100	84	81*	89	99	99	105	98

* $p \leq 0.05$; ** $p \leq 0.01$

Table 37 Selected mean absolute and relative organ weights of mice administered cyflumetofen for 13 weeks (Yoshida, 2004d)

Sex		MALE				FEMALE			
Organ	Dose [ppm]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
Adrenals	0	5.0	100	0.011	100	11.1	100	0.030	100

Sex		MALE				FEMALE			
Organ	Dose [ppm]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
	300	5.0	100	0.011	100	10.3	93	0.028	93
	1000	5.6	112	0.012	109	11.5	104	0.034	113
	3000	5.4	108	0.012	109	11.2	101	0.033	110
	10000	6.6**	132**	0.014**	127*	12.3	111	0.037	123

* p ≤ 0.05; ** p ≤ 0.01

Mortality, clinical signs, body weight development and food consumption were not affected by treatment.

Mean corpuscular haemoglobin concentration was statistically significantly increased (102% of control) in females at 3000 mg/kg food. As the increase was minimal and at 10000 mg/kg food no increase was observed, the increase is considered to be not treatment-related.

Clinical chemistry showed a decreased ASAT at 1000, 3000 and 10000 mg/kg food, decreased ALAT at 1000 and 3000 mg/kg food and decreased blood urea nitrogen at 3000 mg/kg food in males. Control values for ASAT and ALAT were high with high standard deviations in males, almost twice the historical control group mean. After exclusion of three males with exceptionally high values for these two enzymes no statistically significant changes were observed anymore. Moreover, no dose-response relationship was present. As no change in blood urea nitrogen at 10000 mg/kg food was noted, and no dose-response relationship was present, the change at 3000 mg/kg food is not considered to be treatment-related.

Absolute thyroid weight was increased at all dose levels in males only (110, 113, 118 and 123% of control, respectively) with a dose-response relationship. As the increase was not statistically significant, not accompanied by histopathological changes, was only seen in one sex (females showed slight decrease) and in the 28-day study a decrease in male thyroid weight was seen, this finding is considered to be not treatment-related. Absolute and relative adrenal weight was increased at 10000 mg/kg food in males (132 and 127% of control, respectively) and females (111 and 123% of control, respectively).

Macroscopy showed enlarged adrenals in 9/10 males at 10000 mg/kg food. Histopathology revealed diffuse vacuolation of adrenal cortical cells in 2/10 females at 3000 mg/kg food and 7/10 females at 10000 mg/kg food. Diffuse hypertrophy of adrenal cortical cells was seen in one male at 3000 and 10000 mg/kg food.

Based on vacuolation in females and hypertrophy in males of the adrenal cortex at 3000 and 10000 mg/kg food, the NOAEL is set at 1000 mg/kg food (equal to 117 and 150 mg/kg bw/d for males and females, respectively).

13-weeks oral toxicity study in dogs (Nagashima, 2003b)

reference	:	Nagashima, Y., 2003b	exposure	: 13 weeks, gelatine capsule
type of study	:	13-week oral toxicity study	doses	: : 0, 30, 300, 1000 mg/kg bw/d
year of execution	:	2002	vehicle	: None
test substance	:	OK-5101 (batch 01H1; purity 98.4%)	GLP statement	: Yes

route	:	Oral	guideline	:	According to OECD 409
species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**NOAEL**	:	300 mg/kg bw/d

Dogs were given cyflumetofen (purity 98.4%) at dose levels of 0, 30, 300 or 1000 mg/kg bw/d during 13 weeks by gelatine capsule. The results are presented in Table 38. Detailed results on organ weights are given in

Table 39 Mean body weight of dogs administered Cyflumetofen for 28 days

	Males				Females			
Dose level [mg/kg bw]	0	100	300	1000	0	100	300	1000
Body weight [kg]								
- Day 0	8.0	8.1	8.1	8.1	7.2	7.2	7.3	7.2
- Day 28	8.8	9.0	9.1	8.9	7.9	8.0	8.1	7.6
Overall body weight gain [kg]	0.8	0.9	1.0	0.8	0.7	0.8	0.8	0.4

Table 40.

Table 38 Summary table (Nagashima, 2003b)

Dose (mg/kg bw)	0		30		300		1000		dr
	m	f	m	f	m	f	m	f	
Mortality					none				
Clinical signs									
- faeces with test substance					+	++	++	++	m/f
Body weight gain								d	
Food consumption			no treatment-related findings						
Ophthalmoscopy			no treatment-related findings						
Haematology									
- MCHC							ic^1		
- monocytes			ic^2				ic^2		
- prothrombin time						ic^2			
Clin. Chemistry									
- creatinine			dc^2						
- γ-globulin			ic^1				ic^1		
- urea nitrogen								ic^2	
Urinalysis									
- occult blood						$1/4^1$		$1/4^1$	
								$1/4^2$	
- erythrocytes						$1/4^1$		$1/4^1$	
								$1/4^2$	

Dose (mg/kg bw)	0		30		300		1000		dr
	m	f	m	f	m	f	m	f	
Organ weights									
- adrenals							i^a,		
- testes							ic^r,i^a		
- pituitary					$ic^{a,r}$	$i^{a,r}$	$ic^{a,r}$		
- thyroid						ic^a,i^r			
Pathology									
macroscopy									
- adrenal, enlarged	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	
- heart, right atrioventiricular valve, discoloured red foci	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	
- urinary bladder, dark red discoloured foci	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	
microscopy									
adrenal:									
- fine vacuoles, cortical cell, three zonas	0/4	0/4	0/4	0/4	0/4	0/4	4/4	1/4	
-fine vacuoles, cortical cell, zona fasciculata and regularis	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
- large vacuoles, cortical cell, zona fasciculata	0/4	1/4	1/4	2/4	0/4	2/4	3/4	3/4	f

dr	dose related
dc/ic	statistically significantly decreased/increased compared to the controls
d/i	decreased/increased, but not statistically significantly compared to the controls
a/r	absolute/relative
+	present in part of the animals
++	present in all animals
1	week 7
2	week 13

Table 39 Mean body weight of dogs administered Cyflumetofen for 28 days

	Males				Females			
Dose level [mg/kg bw]	0	100	300	1000	0	100	300	1000
Body weight [kg]								
- Day 0	8.0	8.1	8.1	8.1	7.2	7.2	7.3	7.2
- Day 28	8.8	9.0	9.1	8.9	7.9	8.0	8.1	7.6
Overall body weight gain [kg]	0.8	0.9	1.0	0.8	0.7	0.8	0.8	0.4

Table 40 Selected mean absolute and relative organ weights of dogs administered cyflumetofen for 90 days (Nagashima, 2003b)

Sex		MALE				FEMALE			
Organ weight	Dose [mg/kg bw]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
Adrenal (Right)	0	553	100	5.78	100	569	100	6.85	100
	30	617	112	6.47	112	513	90	5.95	87

Sex		MALE				FEMALE			
Organ weight	Dose [mg/kg bw]	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control	Absolute weight [mg]	% of Control	Relative weight [% of b.w.]	% of Control
	300	589	107	6.10	106	611	107	7.00	102
	1000	696	126	**7.54***	130	595	105	7.55	110
Adrenal (Left)	0	522	100	5.47	100	555	100	6.63	100
	30	606	116	6.33	116	487	88	5.59	84
	300	601	115	6.21	114	564	102	6.53	98
	1000	714	137	**7.68***	140	577	104	7.31	110

p ≤ 0.05; ** p ≤ 0.01

No mortality was observed during the study period.

Clinical observations revealed faeces with test substance–like white substance in all animals at 1000 mg/kg bw/d, 1-7 times a week during the treatment period and sporadically in one male and all females at 300 mg/kg bw/d. Body weight gain, from day 1 to 90, was reduced at 1000 mg/kg bw/d in males (64% of control) and females (36% of control). Body weight gain was 86% and 114% of control at 30 and 300 mg/kg bw, respectively, in males and 109% of control at 30 and 300 mg/kg bw/d in females.

Food consumption and ophthalmoscopy were not affected by treatment.

Haematology revealed a slightly increased MCHC relative to control at 1000 mg/kg bw/d in males (102%) in week 7, and statistically significant increases of monocytes at 30 and 1000 mg/kg bw in males in week 13 (167 and 167% of control). As values found were similar to pre-test values, the changes were considered incidental. Statistically significant increase in prothrombin time in females at 300 mg/kg bw/d in week 13 (103% of control) was not considered treatment-related, because pre-treatment values were already higher than controls.

At clinical chemistry the statistically significant changes in creatinine (80% of control), urea nitrogen (142% of control) and γ-globulin (122% of control) were not considered toxicologically relevant as changes were not dose-related or/and values were similar to pre-test values.

Urinalysis showed occult blood and erythrocytes in one female at 300 and 1000 mg/kg bw/d in week 7 and another female at 1000 mg/kg bw/d in week 13. These observations coincided with oestrous bleeding observed during clinical observations and were not considered treatment-related.

Absolute and relative adrenal weight was increased in males at 1000 mg/kg bw/d (right/left: 126/137% and 130/140% of control). Absolute and relative testis weight was increased in males at 1000 mg/kg bw/d (right/left: 131/118% and 133/114% of control). Absolute and relative pituitary weight was increased in females at 300 and 1000 mg/kg bw/d without a dose-relationship and without concomitant histopathological changes and therefore not considered to be treatment-related (141 and 129% for absolute and both 140% for relative compared to control). Absolute and relative thyroid weight was increased at 300 mg/kg bw/d in females (168 and 145% for absolute and 164 and 139% for relative). However, as no increase was seen at 1000 mg/kg bw/d and no dose-relationship the effect is considered to be not toxicologically relevant.

At macroscopy one male at 1000 mg/kg bw/d showed enlarged adrenals (bilateral), dark red foci on the right atrioventricular valve (histopathology: capillary dilation) and dark red foci on the urinary bladder (histopathology: focal mucosal haemorrhage). These latter two findings are normal findings in Beagle dogs. At histopathology the enlarged adrenals showed fine vacuoles in the cortical cells in all three zonas at 1000 mg/kg bw/d in all males (slight to mild). One female showed fine vacuoles in all three zones of the adrenal cortex and one female in the zona fasciculata and reticularis at 1000 mg/kg bw/d (mild). Large vacuoles in the zona fasciculata were noted in 3 males at 1000 mg/kg bw/d, one male at 30 mg/kg bw/d, and in one female in the control group (unilateral), 2 females at 30 and 300 mg/kg bw/d and 3 females at 1000 mg/kg bw/d. All observations of large vacuoles were slight except for two females at 1000 mg/kg bw/d, which were mild. The latter are considered to be treatment-related.

Based on reduced body weight gain, increased adrenal and testis weight and vacuolation of the adrenal cortex at 1000 mg/kg bw/d, the NOAEL is set at 300 mg/kg bw/d.

1-year oral toxicity study in dogs (Nagashima 2004 and 2008)

reference	:	Nagashima, 2004 and 2008 (amendment)	exposure	:	12 months, gelatine capsule
type of study	:	12-months oral toxicity study	doses	:	0, 30, 300, 1000 mg/kg bw/d
year of execution	:	2002/2003	vehicle	:	None
test substance	:	OK-5101 (batch 01H1; purity 98.4%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 452
species	:	Dog, Beagle	acceptability	:	Acceptable
group size	:	4/sex/dose	**LOAEL**	:	30 mg/kg bw/d

In a one year study in dogs, animals were given cyflumetofen (purity 98.4%) 0, 30, 300 or 1000 mg/kg bw/d by gelatine capsule. A summary of the results is presented in Table 41. More detailed results on the adrenal findings are given in Table 42, Table 43 and Table 44.

Table 41 Summary table (Nagashima 2004 and 2008)

Dose (mg/kg bw)	0		30		300		1000		dr
	m	f	m	f	m	f	m	f	
Mortality				none					
Clinical signs - faeces with test substance					+	+	++	++	m/f
Body weight gain				no treatment-related findings					
Food consumption				no treatment-related findings					
Ophthalmoscopy				no treatment-related findings					
Haematology									
- fibrinogen (week 26) (mg/dL)	185	203	269	276*	209*	188	236	222	
- platelets (week 26) (mg/dL)	34.8	26.1	30.8	30.0	33.0	39.3*	28.8	27.4	
- APTT (week 26) (s)	25.4	19.6	20.5	23.2*	20.5	23.5*	20.4	20.4	
- leukocytes week 26) (x10²/µL)	99	81	105	93	117	97	105	135*	

Dose (mg/kg bw)	0		30		300		1000		dr
	m	f	m	f	m	f	m	f	
- stab-neutrophils (week 52) (%)	0.1	0.0	0.0	0.0	0.1	0.4*	0.0	0.1	
Clin. Chemistry									
- glucose (week 52) (mg/dL)	91	97	86	83*	95	91	89	91	
- α2-globulin (week 26) (%)	8.41	5.73	5.61*	5.72	7.26	5.48	7.26	5.80	
- α3-globulin (week 26) (%)	5.49	7.99	7.07	6.14*	6.62	7.00	5.93	5.41*	
- γ-globulin (week 52) (%)	14.97	9.88	12.59	15.38*	14.48	10.88	15.52	11.36	
- triglycerides (week 52) (mg/dL)	23	28	25	20	21	24	16	16	
Urinalysis									
- occult blood						1/4[1]			
- erythrocytes						1/4[1]		1/4[1]	
- sodium (week 26) (mmol/22 h)	14.6	17.4	16.8	11.0	6.3	14.4	3.7*	11.7	
Organ weights									
- adrenals							ic[a,r]	ic[a,r]	
- liver							dc[a], d[r]		
- prostate							dc[a],d[r]		
- pituitary								i[a,r]	
Pathology									
macroscopy									
- adrenal, enlarged	0/4	0/4	0/4	0/4	0/4	0/4	1/4	4/4	
microscopy									
adrenal:									
- cell infiltration, focal	0/4	0/4	0/4	0/4	1/4	2/4	1/4	3/4	f
- fibrosis, interstitial	0/4	0/4	0/4	0/4	0/4	2/4	1/4	3/4	f
- fine vacuoles, cortical cell	0/4	0/4	1/4	2/4	4/4	4/4	3/4	4/4	m/f
- large vacuoles, cortical cell	0/4	4/4	1/4	3/4	2/4	4/4	4/4	4/4	
- degeneration, cortical cell	0/4	0/4	0/4	0/4	1/4	2/4	2/4	1/4	
- pigment laden macrophage	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative
+   present in part of the animals
++   present in all animals
p ≤ 0.05; ** p ≤ 0.01

Table 42 Severity of adrenal vacuolation, 12-months dog (Nagashima 2004 and 2008)

Dose (mg/kg bw)		0		30		300		1000	
		m	f	m	f	m	f	m	f
microscopy									
- fine vacuoles, cortical cell	Slight/minimal	-	-	-	1/4	1/4	2/4	-	-
	Mild	-	-	1/4	1/4	3/4	2/4	1/4	-
	Moderate	-	-	-	-	-	-	2/4	4/4
- large vacuoles, cortical cell	Slight/minimal	-	4/4	1/4	2/4	2/4	1/4	2/4	1/4

Mild	-	-	-	1/4	-	3/4	2/4	2/4
Moderate	-	-	-	-	-	-	-	1/4

Table 43 Background data of the incidence of vacuolation in the adrenal (From 1999 to 2005, Bozo Research Center Inc.)

Sex	MALE	FEMALE
Total number of animals	105	105
Fine vacuole, cortical cell :		
Not remarkable	71 (67.7%)	96 (81.4%)
Minimal	32 (30.5%)	9 (8.6%)
Mild	2 (1.9%)	0
Large vacuole, cortical cell:		
Not remarkable	99 (94.3%)	69 (65.7%)
Minimal	6 (5.7%)	35 (33.3%)
Mild	0	1 (1.0%)

Table 44 Adrenal weight (Nagashima 2004 and 2008)

	Group (mg/kg)	MALE		FEMALE	
		Right	Left	Right	Left
Absolute weight	**Control**	515 mg	507 mg	590 mg	541 mg
	30	112%	110%	101%	105%
	300	110%	113%	118%	111%
	1000	140%**	143%**	168%	164%**
Relative weight	**Control**	4.98 mg%	4.89 mg%	7.24 mg%	6.60 mg%
	30	122%	119%	102%	105%
	300	112%	115%	119%	112%
	1000	147%**	151%**	168%**	166%**

**: $p < 0.01$

No mortality was noted thoughout the study period. Clinical observations frequently revealed faeces with test substance–like white substance in all animals at 1000 mg/kg bw/d during the whole study period and occasionally in animals at 300 mg/kg bw/d. Vomiting, no faeces, soft/mucous/watery faeces and oestrous haemorrhage were noted occasionally in dogs in all groups. Body weight development, food consumption and ophthalmoscopy were not affected by treatment.

Haematology revealed decreased fibrinogen relative to control at 300 mg/kg bw/d in males and increased fibrinogen at 30 mg/kg bw/d in females in week 26. Platelets were increased at 300 mg/kg bw/d and activated partial thromboplastin time was longer at 30 and 300 mg/kg bw/d in females in week 26. An increase of leukocytes was noted at 1000 mg/kg bw/d in females in week 26 and stab-neutrophils were increased at 300 mg/kg bw/d in females in week 52. As these values were similar to pre-test values and/or no change at higher dose levels was noted and the change was only at one point in time, the above changes were considered to be incidental.

At clinical chemistry the statistically significant changes in glucose, α2- and α3- and γ-globulin were not considered toxicologically relevant as changes were not dose-related and only at one point in time. Triglycerides were decreased in males at 1000 mg/kg bw/d in week 26 and 52 relative to control.

Urinalysis showed severe occult blood in one female at 300 mg/kg bw/d and erythrocytes in the same female at 300 mg/kg bw/d (mild) and one female at 1000 mg/kg bw/d (slight) in week 26. These observations coincided with oestrous bleeding observed during clinical observations and were not considered treatment-related. Sodium was decreased relative to the control group, but

similar to pre-test values and variation in values between groups and point in time was large. Therefore, this change was not considered treatment-related.

Absolute and relative liver weight was decreased at 1000 mg/kg bw/d in males (81 and 85% of control, respectively). Absolute and relative adrenal weight was increased at 1000 mg/kg bw/d in males (right/left: 140/143% and 147/151% of control) and females (right/left: 168/164% for absolute and both 168/166% for relative compared to control). Absolute and relative prostate weight was decreased at 1000 mg/kg bw/d (60 and 63% of control, respectively). Absolute and relative pituitary weight was increased in females at 1000 mg/kg bw/d (122% and 121% of control, respectively).

At macroscopy one male and all females at 1000 mg/kg bw/d showed enlarged adrenals (bilateral). At histopathology the adrenals showed fine vacuoles from the zona glomerulosa to the zona reticularis in one male and two females at 30 mg/kg bw/d and in animals at 300 and 1000 mg/kg bw/d with increasing severity at higher dose levels (see Table 42). Large vacuoles were observed in the zona fasciculata and reticularis in one male and three females at 30 mg/kg bw/d, two males and all females at 300 mg/kg bw/d, all animals at 1000 mg/kg bw/d and all females in the control group with some increase in severity towards higher dose levels. The severity of all control animals was slight and in one animal of either sex at 30 mg/kg bw/d the change was mild (other slight). Taking into account that in the historical control data (see Table 43) indicate that minimal (=slight) vacuolation is a common finding in control animals. Also minimal (=slight) to mild fine and/or large vacuolation was seen in a significant number of dogs, the vacuolation in both groups is considered comparable. The vacuolation at 30 mg/kg bw/d is considered to be not an adverse effect, also in view of the absence of clinical signs, adrenal weight change and no degeneration or interstitial fibrosis seen at 30 mg/kg bw/d. However, during the EU review for Annex I inclusion of cyflumetofen the experts in the PRAPeR meeting indicated that there is a monotonic dose response relationship and therefore the use of HCD might not be appropriate. Additionally, the mode of action seems to be related to fat metabolism and supports the relevance of the effect.

The HCD were also discussed. According to the PRAPeR experts, the mild vacuolation is not so common and the HCD are not so convincing. The PRAPeR experts agreed that the adrenal findings (mild vacuoles) at the lowest dose should be considered as an adverse effect (EFSA, 2011).

Slight to mild focal cell infiltration - mainly consisting of lymphocytes - was seen in animals at 300 and 1000 mg/kg bw/d in the zona fasciculata and reticularis. Slight to mild degeneration of cortical cells was noted in one male and two females at 300 mg/kg bw/d and two males and one female at 1000 mg/kg bw/d. The degeneration was characterised by enlarged cortical cells filled with cytoplasmic vacuoles, brown pigment in cytoplasm and karyorrhexis. Slight interstitial fibrosis was seen in two females at 300 mg/kg bw/d and one male and three females at 1000 mg/kg bw/d. Pigment laden macrophages (slight) were noted in one male at 300 and 1000 mg/kg bw/d.

Based on mild vacuolation in adrenals at 30 mg/kg bw/d, the LOAEL is set at 30 mg/kg bw/d, the lowest dose tested.

52-week oral chronic toxicity study in rats, Yoshida, 2004e

reference	:	Yoshida, T., 2004e	exposure	:	Main groups: 24 months, diet Satellite groups: 4, 13, 26 week, diet
type of study	:	1-year chronic toxicity study	doses	:	: 0, 50, 150, 500 or 1500 mg/kg food[1]
year of execution	:	2001-2003	vehicle	:	None
test substance	:	OK-5101 (batch 01D1;	GLP statement	:	Yes

		purity 97.67%)			
route	:	Oral	guideline	:	According to OECD 452
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Acceptable
group size	:	Main groups: 20/sex/dose Satellite groups: 10/sex/dose	**NOAEL**	:	18.8 mg/kg bw/d (M) 23.2 mg/kg bw/d (F)

[1] equal to 0, 1.9, 5.6, 18.8 and 56.8 mg/kg bw/d for males and 0, 2.3, 6.9, 23.3 and 69.2 mg/kg bw/d for females

In a 52 week toxicity study rats were exposed to cyflumetofen (purity 97.67%) at dietary levels of 0, 50, 150, 500 or 1500 mg/kg food. The dose levels were equal to 0, 1.9, 5.6, 18.8 and 56.8 mg/kg bw/d for males and 0, 2.3, 6.9, 23.3 and 69.2 mg/kg bw/d for females. The study was performed according to OECD 452.
Toxicologically relevant effects were noted in rats treated with cyflumetofen at 1500 mg/kg food for 52 weeks (see Table 45 for summary overview). Details on haematology, clinical chemistry and organ weights are given in Table 46, Table 47, Table 48 and Table 49).

One male at the high dose group died at 40 weeks. There were no deaths in any other groups of either sex. Clinical signs, functional observations, body weight development, food consumption and ophthalmoscopy were not affected by treatment.

Effects on erythrocyte count, mean cell haemoglobin, mean cell volume and platelet count were only slight and/or did not occur consistently during the study period and were absent in the semi-chronic toxicity studies and these changes were not considered toxicologically relevant (see Table 46). Fibrinogen decreases were not dose-related and are therefore not considered to be toxicologically significant. The increase in bone marrow is considered to be incidental, as no increased monocyte count was seen in peripheral blood. Other statistically significant changes in bone marrow smears were also considered to be incidental and not toxicologically significant.

Observed changes in blood chemistry were only slight and/or not dose-related and/or only at one point in time, they are not considered to be toxicologically significant (see Table 47 and Table 48).

Urinalysis performed at 13 weeks of treatment or later demonstrated no statistically significant changes in any urinary parameters including volume and pH.

Macroscopy and neoplastic histopathology were not affected by treatment.

Absolute liver weight was slightly increased at 1500 mg/kg food in males in week 4 (107% of control) and relative liver weight was slightly increased in week 4, 13 and 26 (105-107% of control). Histopathology revealed only hepatocytic hypertrophy at 1500 mg/kg food in week 4. Kidney weight was slightly, but significantly increased at 1500 mg/kg food in males in week 13 (105% of control) and females in week 52 (108% and of control). In the absence of histopathological renal findings, increased relative kidney weights are not considered to be toxicologically significant. Absolute and relative adrenal weight was increased in females at 1500 mg/kg food during the whole study (109-115% and 109-117% of control, respectively). This correlates with the diffuse hypertrophy of adrenal cortical cells seen in all females at all scheduled sacrifices. In males at 1500 mg/kg food vacuolation of the adrenal cortex was noted at all scheduled sacrifices. Vacuolation of interstitial gland cells in the ovaries was seen in 3/10 and 4/20 females at 1500 mg/kg food in week 26 and 52, respectively. Organ weights are summarized in detail in Table 49.

No neoplastic lesions were observed in this study up to and including the highest dose tested. Based on vacuolation and hypertrophy of the adrenal cortex for males and females, respectively, and vacuolation of interstitial gland cells in the ovaries, the NOAEL is set at 500 mg/kg food (equal to 18.8 and 23.2 mg/kg bw/d for males and females, respectively).

Table 45 Summary table (Yoshida, 2004e)

Dose (mg/kg food)	0		50		150		500		1500		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	no treatment-related mortality										
Clinical signs	no treatment-related findings										
Functional observations	no treatment-related findings										
Body weight	no treatment-related findings										
Food consumption	no treatment-related findings										
Ophthalmoscopy	no treatment-related findings										
Haematology											
- platelet count									dc^1	dc^2	
- erythrocyte count									ic^2		
- MCH									dc^3		
- MCV									dc^4		
- fibrinogen						dc^4		dc^4	dc^2	dc^4	
Clin. Chemistry											
- BUN			dc^2		dc^2		dc^2		dc^2		
- total protein						dc^2		dc^2		dc^2	
- albumin						dc^2		dc^2	ic^2	dc^2	
- globulin								dc^2		dc^2	
- total cholesterol						dc^2		dc^2		dc^2	
- calcium					dc^5	dc^2	dc^5	dc^2	ic^2/dc^5	dc^2	
- sodium					dc^5	dc^2	dc^5	dc^2	dc^5	dc^2	
- chloride					dc^5	dc^2	dc^5	dc^2	dc^5	dc^2	
Urinalysis	no treatment-related findings										
Organ weights											
- liver									$ic^{a,r}$		
- kidneys									ic^r	ic^r	
- adrenals										$ic^{a,r}$	
Pathology											
macroscopy	no treatment-related findings										
microscopy neoplastic lesions	no treatment-related findings										

Dose (mg/kg food)	0		50		150		500		1500		dr
	m	f	m	f	m	f	m	f	m	f	
non-neoplastic lesions											
liver, hypertrophy											
- week 4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/9	3/10	0/10	
adrenal, cortical cell vacuolation											
- week 4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/9	9/10	0/10	
- week 13	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	0/10	
- week 26	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10	0/10	
- week 52	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	8/19	0/20	
adrenal, cortical cell hypertrophy											
- week 4	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/9	0/10	10/10	
- week 13	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	
- week 26	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0/10	10/10	
- week 52	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20	1/19	20/20	
ovary, vacuolation of interstitial gland cells											
- week 4		0/10		0/10		0/10		0/9		0/10	
- week 13		0/10		0/10		0/10		0/10		1/10	
- week 26		0/10		0/10		0/10		0/10		3/10	
- week 52		0/20		0/20		0/20		0/20		4/20	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative
1   week 4 and 26
2   week 13
3   week 13 and 52
4   week 52
5   week 26

Table 46 Selected haematology findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2004e)

Dose	After Study Week	RBC [10^{12}/L]	MCV [fl]	MCH [fmol]	PLT 10^3/µL	Fib mg/dl	Proe %	Mono %	Reti %	Blas %	Eosi %
MALE											
Control	4	8.18	51.6	18.6	888	236	3.1	0.8	1.0	1.2	3.3
	13	8.62	48.8	17.5	739	224	-	-	-	-	-
	26	9.37	49.5	16.9	822	207	1.4	1.5	0.2	1.2	2.5
	52	9.78	48.1	15.9	807	218	-	-	-	-	-
50 ppm	4	8.17	51.8	18.6	891	244	-	-	-	-	-
	13	8.64	48.7	17.3	737	220	-	-	-	-	-
	26	9.36	49.7	16.8	791	210	-	-	-	-	-
	52	9.71	48.0	15.9	787	215	-	-	-	-	-
150 ppm	4	8.21	51.8	18.4	857	236	-	-	-	-	-
	13	8.63	48.6	17.5	723	219	-	-	-	-	-
	26	9.28	49.4	16.9	808	219	-	-	-	-	-
	52	9.72	48.2	15.9	815	216	-	-	-	-	-
500 ppm	4	8.20	51.7	18.4	857	242	2.3	1.0	0.3*	1.2	2.5*
	13	8.64	48.8	17.5	714	214	-	-	-	-	-
	26	9.33	49.3	16.9	792	209	1.4	1.2	0.3	0.6*	2.6
	52	9.88	47.8	15.7	811	214	-	-	-	-	-

Dose	After Study Week	RBC [10^{12}/L]	MCV [fl]	MCH [fmol]	PLT 10^3/μL	Fib mg/dl	Proe %	Mono %	Reti %	Blas %	Eosi %
1500 ppm	4	8.16	51.7	18.4	841*	232	3.3	1.8**	1.1	1.3	3.2
	13	8.82*	48.5	17.3*	720	209*	-	-	-	-	-
	26	9.35	49.3	16.9	760*	206	1.2	1.3	0.4	1.1	2.7
	52	9.97	47.4*	15.6**	768	217	-	-	-	-	-
FEMALE											
Control	4	7.87	51.1	19.2	793	188	-	-	-	-	-
	13	7.95	51.5	18.7	764	186	1.4	1.8	0.5	0.9	3.9
	26	8.53	53.6	18.4	819	160	-	-	-	-	-
	52	8.54	51.9	17.2	730	173	-	-	-	-	-
50 ppm	4	7.84	51.2	19.0	801	183	-	-	-	-	-
	13	7.82	51.5	18.9	755	180	-	-	-	-	-
	26	8.44	53.7	18.4	809	163	-	-	-	-	-
	52	8.61	52.2	17.3	758	162	-	-	-	-	-
150 ppm	4	7.84	51.4	19.3	823	175	-	-	-	-	-
	13	7.92	51.6	18.6	739	177	-	-	-	-	-
	26	8.50	53.7	18.4	803	158	-	-	-	-	-
	52	8.51	51.9	17.3	781	161*	-	-	-	-	-
500 ppm	4	7.83	51.4	19.0	820	186	-	-	-	-	-
	13	7.92	51.4	18.5	719	180	2.4*	1.5	0.2*	0.9	3.7
	26	8.42	53.5	18.4	792	155	-	-	-	-	-
	52	8.57	52.0	17.3	745	158**	-	-	-	-	-
1500 ppm	4	7.80	51.4	19.3	810	180	-	-	-	-	-
	13	7.91	51.4	18.5	711*	174	1.5	1.2*	0.3	0.7	3.3
	26	8.54	53.3	18.3	800	155	-	-	-	-	-
	52	8.48	52.0	17.2	702	161**	-	-	-	-	-

* p $\leq$ 0.05; ** p $\leq$ 0.01

Table 47 Selected clinical chemistry findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2004e)

Dose	After Study Week	T.Chol mg/dl	TG mg/dl	Ca mg/dl	P mg/dl	Na mEq/dl	K mEq/dl	Cl mEq/dl
MALE								
Control	4	43	46	10.2	7.1	144.9	3.25	105.0
	13	48	58	10.1	5.5	144.3	3.44	107.1
	26	59	61	10.4	4.9	145.4	3.33	108.6
	52	76	77	10.5	4.5	146.1	3.37	107.5
50 ppm	4	44	43	10.0	6.9	144.5	3.28	105.2
	13	46	57	10.3	5.5	144.8	3.39	107.6
	26	53	59	10.2	4.6	144.7	3.40	108.4
	52	85	77	10.5	4.5	146.3	3.38	107.7
150 ppm	4	44	38	10.3	7.2	147.3	3.41	107.0
	13	46	55	10.2	5.3	145.2	3.36	107.7
	26	55	59	**10.2***	4.6	**142.6****	3.35	**106.4****
	52	76	76	10.4	4.3	146.2	3.30	107.9
500 ppm	4	44	39	10.3	7.0	146.7	3.39	107.1
	13	47	58	10.1	5.2	145.1	3.25**	107.9
	26	55	63	**10.1***	4.5	**143.5****	3.25	**107.3****
	52	72	73	10.5	4.3	146.2	3.30	108.1
1500 ppm	4	43	34	10.2	7.0	145.9	3.34	106.4
	13	46	47	**10.3****	5.7	145.2	3.32	107.8
	26	55	66	**10.1****	4.5	**143.1****	3.28	**107.3****
	52	73	69	10.5	4.6	146.2	3.27	107.9
FEMALE								
Control	4	56	12	9.9	6.5	147.3	3.53	112.3

Dose	After Study Week	T.Chol mg/dl	TG mg/dl	Ca mg/dl	P mg/dl	Na mEq/dl	K mEq/dl	Cl mEq/dl
	13	64	18	10.1	5.2	145.9	3.26	110.5
	26	73	27	9.7	4.9	144.2	3.20	108.7
	52	99	52	10.1	3.9	146.1	3.21	109.7
50 ppm	4	54	16	10.0	6.9	146.6	3.50	111.8
	13	67	15	10.0	**4.4***	144.6	3.20	109.4
	26	69	24	9.4	4.1	142.0	3.03	107.5
	52	103	42	10.2	4.0	145.8	3.24	109.6
150 ppm	4	56	11	10.1	6.8	145.7	3.45	110.9
	13	**56****	**13***	**9.4****	4.8	**141.8****	3.15	**108.3***
	26	67	22	9.5	4.4	144.3	3.06	109.3
	52	102	61	10.2	3.9	145.4	3.22	109.0
500 ppm	4	55	13	10.2	6.9	145.5	3.40	110.5
	13	**57***	**12***	**9.2****	**4.4***	**139.9****	3.14	**107.0****
	26	70	30	9.5	4.4	142.0	3.02	**107.0***
	52	96	45	10.1	3.9	145.4	3.16	109.7
1500 ppm	4	54	12	10.0	6.8	147.0	3.38	111.5
	13	**58***	13	**9.5****	4.8	**141.1****	3.20	**107.9***
	26	68	28	9.4	4.3	142.8	3.16	108.5
	52	99	41	10.0	3.5	145.0	3.09	109.3

* p ≤ 0.05; ** p ≤ 0.01

Table 48 Selected blood biochemistry findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2004e)

Dose	After Study Week	Creat mg/dl	BUN mg/dl	TP g/dl	Alb g/dl	Glob g/dl
MALE						
Control	4	0.28	15.1	5.82	4.00	1.82
	13	0.33	18.1	6.24	4.17	2.07
	26	0.37	16.7	6.50	4.34	2.16
	52	0.36	14.9	6.61	4.38	2.22
50 ppm	4	0.29	15.7	5.82	4.01	1.81
	13	0.32	16.6*	6.32	4.18	2.14
	26	0.39*	17.6	6.44	4.30	2.14
	52	0.36	14.8	6.57	4.34	2.23
150 ppm	4	**0.28**	**16.9***	5.97	4.12	1.85
	13	**0.34**	**16.4****	6.37	4.24	2.13
	26	0.38	17.7	6.41	4.27	2.14
	52	0.38	15.3	6.58	4.34	2.24
500 ppm	4	0.28	16.1	5.89	4.07	1.82
	13	0.33	16.1**	6.28	4.20	2.08
	26	0.37	16.5	6.39	4.27	2.12
	52	0.37	15.3	6.62	4.38	2.24
1500 ppm	4	0.28	15.3	5.89	4.07	1.82
	13	**0.32**	**16.5***	6.33	**4.27***	2.06
	26	0.37	17.2	6.42	4.29	2.13
	52	0.36	15.5	6.71	4.42	2.30
FEMALE						
Control	4	0.31	18.3	5.58	3.80	1.78
	13	0.39	19.1	6.10	4.04	2.06
	26	0.41	17.9	6.13	4.01	2.12
	52	0.36	146.6	6.65	4.48	2.17
50 ppm	4	0.29	18.3	5.53	3.74	1.79

Dose	After Study Week	Creat mg/dl	BUN mg/dl	TP g/dl	Alb g/dl	Glob g/dl
	13	0.39	19.0	6.15	4.07	2.08
	26	0.41	16.7	5.93	3.89	2.04
	52	0.37	16.2	6.79	4.57	2.22
150 ppm	4	0.31	19.8	5.71	3.85	1.86
	13	0.38	18.4	**5.73****	**3.76****	1.97
	26	0.43	19.6	5.98	3.93	2.06
	52	0.38	17.7	6.70	4.54	2.16
500 ppm	4	0.30	19.1	5.66	3.83	1.83
	13	0.36	18.8	**5.65****	**3.75****	**1.91****
	26	0.40	18.3	6.12	3.99	2.13
	52	0.36	16.5	6.59	4.43	2.17
1500 ppm	4	0.30	19.1	5.68	3.85	1.83
	13	0.36	18.3	**5.78****	**3.82****	**1.96***
	26	0.39	17.8	5.96	3.91	2.05
	52	0.35	16.0	6.68	4.52	2.16

* $p \leq 0.05$; ** $p \leq 0.01$

Table 49 Selected organ weight findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2004e)

Dose	After Study Week	Wt (g)	% of Control	Relative wt	% of Control	Wt (mg)	% of Control	Relative wt	% of Control
MALE		**Liver**				**Kidney**			
Control	4	5.59	100	2.67	100	1532	100	0.73	100
	13	6.85	100	2.21	100	1828	100	0.59	100
	26	7.36	100	2.04	100	1991	100	0.55	100
	52	8.20	100	2.04	100	2172	100	0.54	100
50 ppm	4	5.55	99	2.71	101	1495	98	0.73	100
	13	6.63	97	2.17	98	1810	99	0.59	100
	26	7.45	101	2.03	100	2010	101	0.55	100
	52	8.33	102	2.05	100	2198	101	0.55	102
150 ppm	4	5.69	102	2.71	101	1526	100	0.73	100
	13	6.92	101	2.20	100	1863	102	0.59	100
	26	7.45	101	2.05	100	1970	99	0.54	98
	52	8.54	104	2.05	100	2216	102	0.53	98
500 ppm	4	5.63	101	2.71	101	1543	101	0.74	101
	13	6.60	96	2.22	100	1768	97	0.59	100
	26	7.52	102	2.07	101	2057	103	0.57	104
	52	8.54	104	2.08	102	2215	102	0.54	100
1500 ppm	4	**5.99***	107	**2.86****	107	1585	103	0.76	104
	13	7.07	103	**2.32****	105	1879	103	**0.62***	105
	26	7.69	104	**2.15****	105	1992	100	0.56	102
	52	8.60	105	2.14	105	2250	104	0.56	104
FEMALE		**Liver**				**Kidney**			
Control	4	3.39	100	2.62	100	959	100	0.74	100
	13	3.51	100	2.13	100	1026	100	0.63	100
	26	3.73	100	2.02	100	1122	100	0.61	100
	52	4.41	100	2.08	100	1264	100	0.59	100
50 ppm	4	3.42	101	2.60	99	983	103	0.75	101
	13	3.53	101	2.10	99	1014	99	0.60	95
	26	3.77	101	2.02	100	1145	102	0.61	100
	52	4.34	98	2.06	99	1271	101	0.60	102
150 ppm	4	3.44	101	2.60	99	990	103	0.75	101
	13	3.41	97	2.09	98	1008	98	0.62	98

Dose	After Study Week	Wt (g)	% of Control	Relative wt	% of Control	Wt (mg)	% of Control	Relative wt	% of Control
	26	3.62	97	2.04	101	1100	98	0.62	102
	52	4.33	98	2.06	99	1277	101	0.61	103
500 ppm	4	3.47	102	2.63	100	990	103	0.75	101
	13	3.47	99	2.07	97	1036	101	0.62	98
	26	3.85	103	2.08	103	1142	102	0.62	102
	52	4.39	100	2.09	100	1295	102	0.62	105
1500 ppm	4	3.53	104	2.67	102	1000	104	0.76	103
	13	3.47	99	2.14	100	1034	101	0.64	102
	26	3.82	102	2.06	102	1155	103	0.62	102
	52	4.52	102	2.16	104	1329	105	**0.64****	108
MALE		**Spleen**				**Adrenal**			
Control	4	505	100	0.24	100	38.0	100	0.018	100
	13	616	100	0.20	100	45.2	100	0.015	100
	26	662	100	0.18	100	44.9	100	0.012	100
	52	683	100	0.17	100	44.1	100	0.011	100
50 ppm	4	496	98	0.24	100	36.1	95	0.018	100
	13	624	101	0.20	100	44.2	98	0.015	100
	26	653	99	0.18	100	43.6	97	0.012	100
	52	711	104	0.18	106	60.0	136	0.015	136
150 ppm	4	509	101	0.24	100	37.2	98	0.018	100
	13	626	102	0.20	100	41.6**	92	0.013**	87
	26	655	99	0.18	100	42.8	95	0.012	100
	52	687	101	0.17	100	47.4	107	0.012	109
500 ppm	4	493	98	0.24	100	37.0	97	0.018	100
	13	575*	93	0.19	95	44.0	97	0.015	100
	26	674	102	0.19	106	43.4	97	0.012	100
	52	743	109	0.18	106	45.2	102	0.011	100
1500 ppm	4	500	99	0.24	100	38.9	102	0.0190	106
	13	612	99	0.20	100	45.5	101	0.015	100
	26	642	97	0.18	100	45.2	101	0.013	108
	52	706	103	0.18	106	47.7	108	0.012	109
FEMALE		**Spleen**				**Adrenal**			
Control	4	350	100	0.27	100	44.3	100	0.034	100
	13	394	100	0.24	100	46.5	100	0.028	100
	26	419	100	0.23	100	47.6	100	0.026	100
	52	448	100	0.21	100	51.0	100	0.024	100
50 ppm	4	367	105	0.28	104	43.7	99	0.033	97
	13	385	98	0.23	96	45.7	98	0.027	96
	26	409	98	0.22	96	48.0	101	0.026	100
	52	428	96	0.20	95	53.3	105	0.025	104
150 ppm	4	355	101	0.27	100	44.4	100	0.033	97
	13	376	95	0.23	96	45.0	97	0.028	100
	26	409	98	0.23	100	49.0	103	0.028	108
	52	450	100	0.22	105	50.8	100	0.024	100
500 ppm	4	363	104	0.27	100	44.8	101	0.034	100
	13	384	97	0.23	96	48.3	104	0.029	104
	26	420	100	0.23	100	50.3	106	0.027	104
	52	437	98	0.21	100	54.5	107	0.026	108
1500 ppm	4	356	102	0.27	100	49.1**	111	0.037*	109
	13	382	97	0.24	100	50.7	109	0.031*	111
	26	427	102	0.23	100	54.3**	114	0.029**	112
	52	448	100	0.21	100	58.8**	115	0.028**	117

* p ≤ 0.05; ** p ≤ 0.01

52-week oral chronic toxicity study in rats (Yoshida, 2012)

reference	:	Yoshida, T., 2012	exposure	:	Main groups: 12 months, diet
					Satellite groups: 6 months, diet
type of study	:	1-year chronic toxicity study	doses	:	: 0, 6000 mg/kg food[1]
year of execution	:	2011-2012	vehicle	:	None
test substance	:	OK-5101 purity 97.82%)	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 452
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Acceptable
group size	:	Main groups: 20/sex/dose Satellite	**NOAEL**	:	<250 mg/kg bw/d (M)
		groups: 30/sex/dose			<319 mg/kg bw/d (F)

[1] corresponding to 250 mg/kg bw/d for males and 319 mg/kg bw/d for females

In a 52 week long-term toxicity study rats were exposed to cyflumetofen (purity 97.82%) at dietary levels of 0 or 6000 mg/kg food. The dose levels were equal to 0 and 250 mg/kg bw/d for males and 0 and 319 mg/kg bw/d for females. Satellite groups were sacrificed after 4, 13 and 26 weeks of administration. The study was performed according to OECD 452. Toxicologically relevant effects were noted in rats treated with cyflumetofen at 6000 mg/kg food (see Table 51 to Table 54).

There were no significant changes in mortality rate in any treated group of either sex when compared to controls. At 6000 mg/kg food, body weight of females was significantly reduced throughout most of the treatment period. Females at 6000 mg/kg food showed an increased incidence of soiled fur. In the 6000 ppm group, males showed a significant increase in food consumption at week 13 and females showed a significant decrease in food consumption at week 9.

Treated animals of both sexes showed significant decreases in platelet count (PLT) and fibrinogen concentration (Fib) and a significant extension in prothrombin time (PT) after 4 weeks of treatment. Similar changes in these parameters were detected in the treated males after 13 and/or 52 week of treatment, together with a significant extension in activated partial thromboplastin time after 4, 13, and 52 weeks of treatment (see Table 51). In blood proteins, the treated males showed significant increases in total protein, albumin, and albumin/globulin ratio (A/G ratio) after 13, 26, and/or 52 week of treatment. Females showed a treatment related significant increase in blood urea nitrogen after 13, 26, and/or 52 week of treatment (see Table 52). In organ weight measurement, the treated males and females showed significant increases in absolute and/or relative weights of the liver, kidneys and adrenals (see Table 53).

Histopathologically, the treated females showed a significant increase in the incidence of vacuolation of interstitial gland cell in the ovary at all examination periods. All treated females also showed a diffuse hypertrophy of the adrenal cortex. In treated males, there was a high incidence of vacuolation of the adrenal cortex. The treated males showed a significant increase in the incidences of focal atrophy of acinar cell in the pancreas and hyperplasia of interstitial cell in the testis at the examination (only at the 52 week examination). Histopathological findings are summarized in Table 54. Historical control data on the incidence of acinar cell atrophy in the pancreas and interstitial cell hyperplasia in the test are given in Table 55.
Based on the observed effects on the adrenal, ovary, pancreas (males) and testis, the LOAEL is set at 6000 ppm (equal to 250 and 319 mg/kg bw/d for males and females, respectively). The prior 1-yr rat study at lower doses (Yoshida, 2004e) establishes a NOAEL for all of these effects at 1500 ppm (56.8 and 69.2 mg/kg bw in males and females respectively).

Table 50 Mean body weight of rats administered Cyflumetofen for 1 year (main group)

	Males		Females	
	0	6000	0	6000
Body weight [g]				
Day 0	107	107	87	87
Week 13	310	307	174	168*
Week 52	414	404	214	201**
Wk 52 Body Weight (% of Control)	100	97.5	100	93.9
Overall body weight gain [g]				
Week 13	203	200	87	81
Wk 13 Gain (% of control) [#]	100	98.5	100	93.1
Week 52	307	297	127	114
Wk 52 Gain (% of control) [#]	100	96.7	100	89.8

[#] Values may not calculate exactly due to rounding of mean values

* $p \leq 0.05$; ** $p \leq 0.01$

Table 51 Selected hematology findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2012)

Dose	After Study Week	RBC [10^{12}/L]	MCV [fl]	MCH [fmol]	PLT 10^3/μL	Fib mg/dl	Retics %	PT sec
MALE								
Control	4	8.77	55.2	17.4	968	224	186	13.0
	13	8.76	51.8	16.6	796	196	148.6	16.8
	26	8.68	50.0	16.8	700	237	141.5	15.5
	52	9.19	50.6	16.2	749	262	182	13.1
6000 ppm	4	8.75	55.3	17.3	916**	203**	180.9	17.8**
	13	8.76	51.3*	16.5	736**	179**	146.4	18.9*
	26	8.88*	49.5	16.6	691	226	141.5	16.6
	52	9.33	50.0*	16.0	725	244	168.2*	14.5**
FEMALE								
Control	4	8.49	54.6	17.7	892	203	147.4	10.2
	13	8.14	54.1	18.0	741	162	139.5	10.3
	26	7.84	54.0	18.3	680	177	172.2	10.2
	52	8.33	54.6	17.7	656	167	183.0	10.0
6000 ppm	4	8.41	54.5	17.7	809**	179**	130.1	10.5*
	13	8.08	54.1	18.0	721	152	141.0	10.5
	26	7.89	53.8	18.1*	675	168	146.5*	10.2
	52	8.28	54.2	17.5	670	160	184.0	10.0

* $p \leq 0.05$; ** $p \leq 0.01$

Table 52 Selected blood biochemistry findings in rats administered cyflumetofen for 1 year (group means) (Continued from prior table) (Yoshida, 2012)

Dose	After Study Week	Glob mg/dl	T.Chol mg/dl	TG mg/dl	T.Bil mg/dl	K mEq/L
MALE						
Control	4	1.66	43	56	0.03	3.59
	13	1.90	43	59	0.04	3.34
	26	2.13	54	62	0.04	3.52
	52	2.38	76	92	0.05	3.48

Dose	After Study Week	Glob mg/dl	T.Chol mg/dl	TG mg/dl	T.Bil mg/dl	K mEq/L
6000 ppm	4	1.62	39**	34**	0.03	3.67
	13	1.85	45	58	0.03	3.42
	26	2.07	53	56	0.04	3.41
	52	2.36	71	73*	0.05*	3.39
FEMALE						
Control	4	1.45	50	16	0.04	3.57
	13	1.67	58	22	0.05	3.27
	26	1.71	73	28	0.06	3.23
	52	1.90	94	30	0.07	3.33
6000 ppm	4	1.31**	46*	12**	0.03	3.47
	13	1.61	57	19	0.05*	3.14*
	26	1.70	68	21*	0.05	3.19
	52	1.80	94	33	0.06**	3.42

* p≤ 0.05; ** p≤ 0.01

Table 53 Selected organ weight findings in rats administered cyflumetofen for 1 year (group means) (Yoshida, 2012)

Dose	After Study Week	Wt [mg] or [g]	% of Control	Relative wt	% of Control	Wt [mg] or [g]	% of Control	Relative wt	% of Control
		Liver [g]				**Kidney [mg]**			
MALE									
Control	4	5.71	100	2.84	100	1467	100	0.73	100
	13	6.77	100	2.26	100	1821	100	0.61	100
	26	7.28	100	2.06	100	2031	100	0.58	100
	52	8.00	100	2.01	100	2170	100	0.54	100
6000 ppm	4	**6.36****	**111**	**3.17****	**112**	**1564****	**107**	**0.78****	**107**
	13	**7.62****	**113**	**2.60****	**115**	**1916****	**105**	**0.66****	**108**
	26	**8.22****	**113**	**2.34****	**114**	**2157***	**106**	**0.61****	**105**
	52	**8.84****	**111**	**2.26****	**112**	**2280***	**105**	**0.58****	**107**
FEMALE									
Control	4	3.61	100	2.79	100	1032	100	0.80	100
	13	3.56	100	2.20	100	1081	100	0.67	100
	26	3.89	100	2.18	100	1191	100	0.67	100
	52	4.37	100	2.14	100	1336	100	0.65	100
6000 ppm	4	3.67	102	**2.98****	**107**	1052	102	**0.86****	**108**
	13	**3.90****	**110**	**2.41****	**110**	**1163****	**108**	**0.72****	**107**
	26	4.00	103	**2.37****	**109**	1234	104	**0.73****	**109**
	52	4.55	104	**2.36****	**110**	1361	102	**0.71****	**109**
		Spleen [mg]				**Adrenal [mg]**			
MALE									
Control	4	496	100	0.25	100	44.2	100	0.022	100
	13	586	100	0.20	100	45.3	100	0.015	100
	26	645	100	0.18	100	44.8	100	0.013	100
	52	674	100	0.17	100	46.6	100	0.012	100
6000 ppm	4	495	100	0.25	100	**48.7***	**110**	**0.024***	**109**
	13	584	100	0.20	100	**56.4****	**125**	**0.019****	**127**
	26	649	101	0.18	100	**52.9****	**118**	**0.015****	**115**
	52	676	100	0.17	100	**58.4****	**125**	**0.015****	**125**
FEMALE									
Control	4	356	100	0.28	100	48.1	100	0.037	100
	13	388	100	0.24	100	48.1	100	0.030	100
	26	427	100	0.24	100	48.1	100	0.027	100

Dose	After Study Week	Wt [mg] or [g]	% of Control	Relative wt	% of Control	Wt [mg] or [g]	% of Control	Relative wt	% of Control
	52	433	100	0.21	100	53.0	100	0.0026	100
6000 ppm	4	351	99	**0.29***	**104**	**73.2****	**152**	**0.060****	**162**
	13	410	106	0.25	104	**83.2****	**173**	**0.052****	**173**
	26	408	96	0.24	100	**77.0****	**160**	**0.046****	**170**
	52	429	99	**0.22***	**105**	**80.5****	**152**	**0.042****	**162**

* p≤ 0.05; ** p≤ 0.01

Table 54 Incidence of selected histopathological findings in rat administered cyflumetofen for 1 year (Yoshida, 2012)

Organ / Lesion	Wk	Dose Group (ppm)			
		Male		Female	
		Control	6000	Control	6000
Adrenal: Vacuolation, cortical cell, diffuse	4	0/10	10/10**	-	-
	13	0/10	10/10**	-	-
	26	0/10	10/10**	-	-
	52	1/20	19/20**	-	-
Adrenal: Hypertrophy, cortical cell, diffuse	4	-	-	0/10	10/10**
	13	-	-	0/10	10/10**
	26	-	-	0/10	10/10**
	52	-	-	0/10	20/20**
Ovary: Vacuolation, interstitial gland cell	4	-	-	0/10	10/10**
	13	-	-	0/10	10/10**
	26	-	-	0/10	10/10**
	52	-	-	0/20	11/20**
Pancreas: Atrophy, acinar cell, focal	4	0/10	0/10	0/10	0/10
	13	0/10	0/10	0/10	0/10
	26	2/10	3/10	0/10	0/10
	52	4/20	14/20**	0/20	1/20
Testis: Hyperplasia, interstitial cell	4	0/10	0/10	-	-
	13	0/10	0/10	-	-
	26	0/10	0/10	-	-
	52	6/20	19/20**	-	-

Data were statistically analysed by Fisher's exact probability test

* p≤ 0.05; ** p≤ 0.01

Table 55 Historical control incidence of focal atrophy of acinar cell in the pancreas and hyperplasia of interstitial cell in the testis at the conducting laboratory (2004~2011) Male (After 52 weeks of treatment)

Organs and lesions	Study ID	A	B	C	D	E	F	G	H
	Year	2004	2004	2007	2007	2008	2008	2010	2011
	N	25	25	20	20	20	20	20	20
Pancreas: Atrophy, acinar cell, focal		4	1	0	4	2	0	2	5
Testis: Hyperplasia, interstitial cell		0	0	2	0	2	1	0	9

4.7.1.2 Repeated dose toxicity: inhalation

Inhalation studies were not submitted and are not considered required: the vapour pressure of cyflumetofen $<< 10^{-2}$ Pa and the substance needs no classification for acute inhalation toxicity. Furthermore, the respiratory exposure is low.

4.7.1.3 Repeated dose toxicity: dermal

28-days dermal toxicity study in rats (Buesen, 2010)

Wistar rats were administered cyflumetofen (purity 98.4%, vehicle 1% CMC) by the dermal route at dose levels of 0, 100, 300 or 1000 mg/kg bw/d during 4 weeks. Clinical examinations did not indicate any signs of general systemic toxicity. No mortality was observed in this study.In addition, no local dermal irritation was observed. Body weight development, food consumption and ophthalmoscopy was not affected by treatment. During the functional observational battery as well as measurement of motor activity, no signs of neurotoxicity were observed. Hematology, clinical chemistry and urinalysis were not affected by trement. The absolute weight of the kidneys was increased slightly in the 300 and 1000 mg/kg bw doses with the males. There was no histopathological correlate, and this finding was considered incidental. The relative liver weight was increased statistically significantly in the 1000 mg/kg bw dose with the males. As there was no histopathological correlate, this finding was regarded as incidental. All macroscopic findings observed at necropsy were considered to be incidental and spontaneous in nature and were not related to treatment. All histopathological findings occurred either individually or were biologically equally distributed over the control group and the treatment groups. They were considered to be incidental or spontaneous in origin and without any relation to treatment. Therefore, under the conditions of the present study, the no observed adverse effect level for systemic and local effects (NOAEL) was at least 1000 mg/kg body weight/day for male and female rats.

4.7.1.4 Repeated dose toxicity: other routes

No studies were submitted.

4.7.1.5 Human information

No human data available.

4.7.1.6 Other relevant information

Mechanistic studies are summarised in section 4.12 Specific investigations.

4.7.1.7 Summary and discussion of repeated dose toxicity

Short term oral exposure to cyflumetofen was studied in rat, mouse and dog. Increased adrenal weight and vacuolation of adrenal cortical cells was observed in all three species investigated, with rat being the most sensitive species. In rat these observations were accompanied by increased liver weight, hypertrophy of hepatocytes and ovarian interstitial cell vacuolation, with a NOAEL of 37.6 and 40.8 mg/kg bw/day in males and females, respectively.

Vacuolation of adrenal cortical cells was also the effect triggering the lowest endpoint in rat, mouse and dog after semi-chronic oral administration of cyflumetofen. For this effect dogs showed a

LOAEL of 30 mg/kg bw/day (mild vacuolation) and rats showed the lowest NOAEL of 16.5 and 19.0 mg/kg bw/day in males and females, respectively.

In long term toxicity studies and carcinogenicity studies with rat (see for carcinogenicity studies section 4.10.1.1), the overall NOAEL for vacuolation and hypertrophy of the adrenal cortex was 16.5 and 20.3 mg/kg bw/day in males and females, respectively.

A 28-day oral mechanistic study in rat was performed to elucidate the mechanism(s) for the observed effects on adrenals and ovary. The results show that the observed cyflumetofen-induced vacuolation of adrenal cortical cells and the vacuolation of the interstitial cells of the ovary are likely due to cholesterol deposition as a result of a reduction in hormone sensitive lipase.

Overall, repeated oral administration of cyflumetofen caused vacuolation and hypertrophy of adrenal cortical cells in the species investigated (rat, mouse, dog), with an overall NOAEL in rats of 16.5 and 19.0 mg/kg bw/day in males and females, respectively, observed in semi chronic and chronic studies.

In the 4-week dermal toxicity study, no adverse effects were seen and the NOAEL for local and systemic effects was established at 1000 mg/kg bw/day.

4.8   Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1   Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Increased adrenal weight and vacuolation of adrenal cortical cells was observed in all three species investigated, with rat being the most sensitive species. In rat these observations were accompanied by increased liver weight, hypertrophy of hepatocytes and ovarian interstitial cell vacuolation. Vacuolation of adrenal cortical cells was also the effect triggering the lowest endpoint in rat, mouse and dog after semi-chronic oral administration of cyflumetofen. In long term toxicity studies and carcinogenicity studies with rat also vacuolation and hypertrophy of the adrenal cortex was seen. In a 28-oral study in rats (cf 4.7.1.1.) the vacuoles seen in the adrenals and ovaries were shown to consist of lipid droplets. The vacuolation of adrenal cortical cells and vacuolation of interstitial ovary cells after repeated exposure to cyflumetofen might be due to cholesterol and cholesterylester deposition as a result of a reduced activity of hormone-sensitive lipase (HSL). However, this has not been proven and other mechanisms cannot be excluded. For example, steroidogenesis occurs partly in the mitochondria and requires energy in the form of NADPH. Cyflumetofen acts on the target species via an effect on the mitochondria. For mammals such effect only occurs at much higher dose levels. (Hayashi et al., 2013). An effect on steroidogenesis is also consistent with the results of the in vitro steroidogenesis assay (section 4.12.1.3) and the possible changes in hormonal levels in the 2-generation study.

In summary in the repeated dose studies, the effects seen were vacuolation in adrenals and ovary, increased adrenal and liver weight and hypertrophy of hepatocytes.

Considering the adrenal effects, vacuolation and increased adrenal weight were seen across the available studies. In a 28-day oral mechanistic study in rats (cf. 4.12.1.3) decreased levels of HSL in the presence of adrenal weight increase and adrenal cortical cell vacuolation were not accompanied by a change in serum ACTH (adrenocorticotropic hormone) or corticosterone for either sex, indicating that adrenal function was not affected.

The vacuolation of interstitial ovary cells, was not accompanied by any further histopathological change or functional disturbance of the ovary in studies with prolonged exposure or in reproductive toxicity studies.

The increase in liver weight and hypertrophy of hepatocytes was not accompanied by any further functional changes in liver, e.g. no relevant changes in clinical biochemistry were noted.

Observed effects in adrenal, ovary and liver are not considered significant toxic effects since, these effect did not result in organ damage, necrosis, fibrosis or granuloma formation, organ dysfunction or cell death, even at longer exposure times (chronic exposure duration).

Overall, the vacuolation or hypertrophy of the adrenal cortex *per se* is considered a treatment related, but not adverse effect. There is no indication of dysfunction of the adrenal glands even after lifetime exposure.

4.8.2   Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Classification for repeated dose toxicity depends on the type of effects and the dose at which the effects are observed. The CLP criteria state that STOT-RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. In this context 'significant' means changes which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. 'Severe' effects are generally more profound or serious than 'significant' effects and are of a considerably adverse nature which significantly impact on health.

Classification in Category 1 is applicable, when significant toxic effects observed in a 90-day repeated oral dose toxicity study (rat) are seen at or below 10 mg/kg bw/day.

Classification in Category 2 is applicable, when significant toxic effects observed in a 90-day repeated-dose oral toxicity study in rat are seen to occur in case the limit value is greater than 10 and lower or equal to 100 mg/kg bw/day.

The main target of cyflumetofen after repeated exposure is the adrenals, as observed in rat, mouse and dog. The effect triggering the lowest endpoint was vacuolation of the adrenal cortical cells, which was observed in sub-acute, semi chronic and chronic toxicity studies.

For dietary studies, the overall LOAEL for these effects was set in a 13-week oral study in rats, in which the LOAEL was set at 54.5 mg/kg bw/day for males and 62.8 mg/kg bw/day, with similar LOAELs from a 1-year study in rat (56.8 mg/kg bw/day in males and 69.2 mg/kg bw/day in females). In a 1-year study in dogs (capsules), a LOAEL was set at < 30 mg/kg bw/day.

In the chronic studies, the LOAEL for vacuolation of adrenal cortical cells was similar to the LOAEL observed in semi chronic studies, indicating no increase of the effect after prolonged exposure. Besides, in the chronic studies no additional effects indicative for toxicity were observed related to the adrenal cortex. Moreover, considering all toxicity data on cyflumetofen, significant or severe toxicity related to the vacuolation of the adrenal cortical cells was not observed in any species studied. Furthermore, for the ovary vacuolation, no significant or severe toxicity related to this observation was observed in any further study with cyflumetofen. In addition, no significant effect other than increased liver weight or liver hypertrophy was noted in any of the studies. As all available toxicity data on cyflumetofen give no indication for functional disturbance or morphological changes which are toxicologically relevant, and there is no indication of significant impact on health in any of the toxicity studies, it can be concluded that the cyflumetofen-induced vacuolation of adrenal cortical cells, vacuolation of the ovary cells or increased liver weight, is not

related to significant or severe toxicity. Therefore, the effects observed in repeated dose toxicity studies are considered not relevant for classification as STOT RE 2.

4.8.3   Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

In the absence of any indication for functional disturbance, toxicologically relevant morphological changes or significant impact of health in any of the toxicity studies, the observed adrenal, ovary and liver effects do not meet the criteria for classification for repeated dose toxicity. Therefore, classification for STOT RE is not required.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

In the CLH report, the DS summarised:

- One oral (dietary) 28 days and 13 weeks repeated dose toxicity (RDT) studies in CD-1 mice,

- six oral (dietary) RDT studies in Fischer F344 rats (ranging in duration from 14 days to 1 year),

- and three RTD studies in beagle dogs (oral, gelatine capsules; 28 days, 13 weeks and 12 months)

In addition, non-neoplastic findings from the carcinogenicity studies were considered for STOT RE. All studies were described as compliant with the relevant OECD guidelines, except a 28 day non-GLP compliant range finding study in rats. Also, the 14 day study in rats was OECD TG but not GLP compliant. The doses used were up to 1000 mg/kg bw/day in rats and dogs and up to 1500 mg/kg bw/day in mice.

Increased adrenal weight and vacuolation of adrenal cortical cells was observed in all three species investigated. In the rat, the most sensitive species tested, these observations were accompanied by increased liver weight, hypertrophy of hepatocytes and ovarian interstitial cell vacuolation. Results from a 28 days oral mechanistic study in rats, which was designed to elucidate the mechanism(s) for the observed effects on adrenals and ovary, indicated that the observed vacuolation in these organs was probably due to intracellular cholesteryl ester accumulation and deposition as a result of a reduction in hormone sensitive lipase and its ability to make cholesterol available from cholesteryl esters for steroid synthesis.

The DS discussed in the CLH report the relevance of these findings for classification. The DS noted that although increased adrenal weight and adrenal cortical cell vacuolation was seen in three species in studies of various durations, the vacuolation was not accompanied by a change in (basal) serum adrenocorticotropic hormone or corticosterone in either sex, indicating that adrenal function was not affected. Furthermore, the LOAEL for vacuolation of adrenal cortical cells was similar in rats regardless of the duration of the treatment, indicating no increase of the effect after prolonged exposure.

The vacuolation of interstitial ovary cells was not accompanied by any other histopathological

changes or functional disturbance of the ovaries.

The increase in liver weight and hypertrophy of hepatocytes was not accompanied by relevant changes in clinical biochemistry.

The DS concluded that the observed effects in adrenal, ovary and liver, even if they were treatment-related, were not sufficiently adverse to warrant classification.

A further non-GLP but OECD TG 410 compliant 28 days dermal RDT study was conducted in Wistar rats at doses up to 1000 mg/kg bw/day. No treatment related effects were observed.

No RDT studies were conducted via the inhalation route.

In addition, a 90 days (non-GLP and non-OECD TG compliant, Buesen 2011) oral neurotoxicity RDT study in Wistar rats was summarised. No adverse neurobehavioral effects or test substance-related effects were observed in the neurohistopathology investigation in males or females at up to 353 mg/kg bw/day.

In a 28 day oral RDT (non-GLP and non-OECD TG compliant) immunotoxicity study in female Wistar rats, all animals were immunised 6 days before blood sampling and necropsy using intraperitoneally administered sheep red blood cells. Administration of cyflumetofen did not reveal any signs of immunotoxicity at doses up to 349 mg/kg bw/day.

Based on results from the above studies, the DS concluded that the findings observed did not meet the criteria for classification for repeated dose toxicity and proposed no classification for STOT RE.

Comments received during public consultation

One MSCA agreed with the DS proposal for no classification for STOT RE. Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal to not classify cyflumetofen for STOT RE.

Assessment and comparison with the classification criteria

Summary of all the short-term and long-term study data with respect relevant effects

In general, the effects observed below the guidance trigger values are not considered severe in the context of organ dysfunction and tissue damage. All values are relative to controls.

Table: Summary of relevant NOAELs and LOAELs and effects from repeated dose toxicity studies compared with <u>STOT RE 2</u> trigger values.

Study	Criteria for STOT RE 2	NOAEL	LOAEL	Effects at LOAEL (Male/Female)	Reference
		mg/kg bw/d			
Oral studies					
Rat F344 14-day (dietary)	≤ 600	M: --	**101**	↑ liver wt rel. (+10%*/--) ↑ adrenal wt abs. (--/+36%*) ↑ adrenal wt rel. (+25%*/+36%*) ↑ vacuolation of adrenal cortical	Sakai, 2001
		F: --	**105**		

				↑ cells (0/6 vs [6/6]/[6/6]) ↑ vacuolation of interstitial gland cells ovary (0/6 vs [--]/[6/6])	
Rat F344 26-day (dietary)	≤ 300	M: 43	**128**	↑ cortical cell vacuolation ([0/5] vs [3/5]/[3/5]) ↑ adrenal wt abs. (--/+34%**) ↑ adrenal wt rel. (--/+36%**)	Buesen, 2010
		F: 46	**132**		
Rat F344 28-day (dietary)	≤ 300	M: 37.6	**75.1**	↑ adrenal wt rel (--/+15%*) ↓ triglycerides (-20%**/--) ↑ vacuolation of adrenal cortical cells (0/6 vs [6/6]/[6/6]) ↑ vacuolation of interstitial cells ovary (0/6 vs [--]/[2/6])	Yoshida, 2004a
		F: 40.8	**79.8**		
Mouse CD-1 28-day (dietary)	≤ 300	M: 135	663	↑ adrenal weight ↑ vacuolation and hypertrophy of adrenal cortical cells ↑ hyperplasia of adrenal subcapsular cells	Yoshida, 2004b
		F: 150	763		
Dog 28-day (capsule)	≤ 300	M: 100	**300**	↑ vacuolation of adrenal cortical cells (0/3 vs [2/3]/[3/3]) ↑ adrenal wt abs (+16%/+28%) ↑ adrenal wt rel (+11%/+24%)	Nagashima, 2003a
		F: 100	**300**		
Rat F344 90-day (dietary)	≤ 100	M: 16.5	**54.5**	↑ vacuolation of adrenal cortical cells (0/10 vs [6/10]/[--]) ↑ adrenal cortex hypertrophy (0/10 vs [--]/[10/10])	Yoshida, 2004c
		F: 19.0	**62.8**		
Mouse CD-1 90-day (dietary)	≤ 100	M: 117	348	↑ vacuolation of adrenal cortical cells ↑ adrenal cortex hypertrophy	Yoshida, 2004d
		F: 150	447		
Dog 90-day (capsule)	≤ 100	M: 300	1000	↓ body weight gain ↑ adrenal and testis weight ↑ vacuolation adrenal cortex	Nagashima, 2003b
		F: 300	1000		
Dog 1-year (capsule)	≤ 25	M: --	30	↑ adrenal findings (mild vacuoles)	Nagashima, 2004 and 2008
		F: --	30		
Rat F344 1-year (dietary)	≤ 25	M: 18.8	56.8	↑ vacuolation of adrenal cortical cells ↑ adrenal cortex hypertrophy ↑ vacuolation of interstitial cells ovary	Yoshida, 2004e
		F: 23.2	69.2		
Rat F344 1-year (dietary)	≤ 25	M: --	250	↑ testis: interstitial cell hyperplasia (6/20 vs 19/20) ↑ M: vacuolation of adrenal cortical cells (1/20 vs 19/20) ↑ M: Pancreas: atrophy, acinar cell, focal (4/20 vs 14/20) ↑ vacuolation of interstitial cells ovary (0/20 vs 11/20)	Yoshida, 2012
		F: --	319		
Dermal studies					
Rat Wistar 28-day	≤ 600	> 1000	--	No adverse effects	Buesen R. *et al.* 2010a

-- Not relevant (generally less than 10% of controls)
N.B. If there is no value for a particular effect it was because the data was not supplied in the CLH report or the LOAEL is outside of the upper bound trigger values for STOT-RE 2.

* p ≤ 0.05; ** p ≤ 0.01

Major Target Organs

The major target organ in rats, mice, and dogs following oral administration of cyflumetofen is the adrenal gland. The lesions are characterized by vacuolation and hypertrophy of the adrenal cortical cells and there is much speculation over the mode of action for this effect with limited evidence for the mechanism(s) of action involved. The rat is the most sensitive species. In rat these observations were accompanied by increased liver weight, hypertrophy of hepatocytes and ovarian interstitial cell vacuolation. Vacuolation of adrenal cortical cells was also the effect triggering the lowest endpoint in rats, mouse and dog after semi-chronic oral administration of cyflumetofen. Long term toxicity studies and carcinogenicity studies with rats also showed extensive vacuolation and hypertrophy of the adrenal cortex.

The available evidence indicates a disruption to steroid metabolism and cholesterol utilisation in the adrenals (See BD). The morphological effects in the adrenals (diffuse vacuolation of cortical cells; subcapsular cell hyperplasia; with occasional and slight deposition of brown pigment in the cortico-medullary junction) are similar to what is seen in hormone-sensitive lipase (HSL) deficient mice (HSL$^{-/-}$). However, not only do we have more profound morphological changes than those indicated above occurring in HSL$^{-/-}$ mice, but there is also hypertrophy of brown and white adipose tissue (due to impairment of triglyceride hydrolysis) along with more severe effects such as impaired or blunted corticosterone secretion following acute adrenocorticotropic hormone (ACTH) stimulation and oligospermia, leading to infertility in affected male mice (Osuga et al., 2000; Li et al., 2002). An effect on steroidogenesis is also consistent with the results of the in vitro steroidogenesis assay and the possible changes in hormonal levels in the 2-generation study.

Figure: 2-year rat carcinogenicity dietary study (Takahashi, 2013). Adrenal sections taken from control (a) and high dose (b) female rats
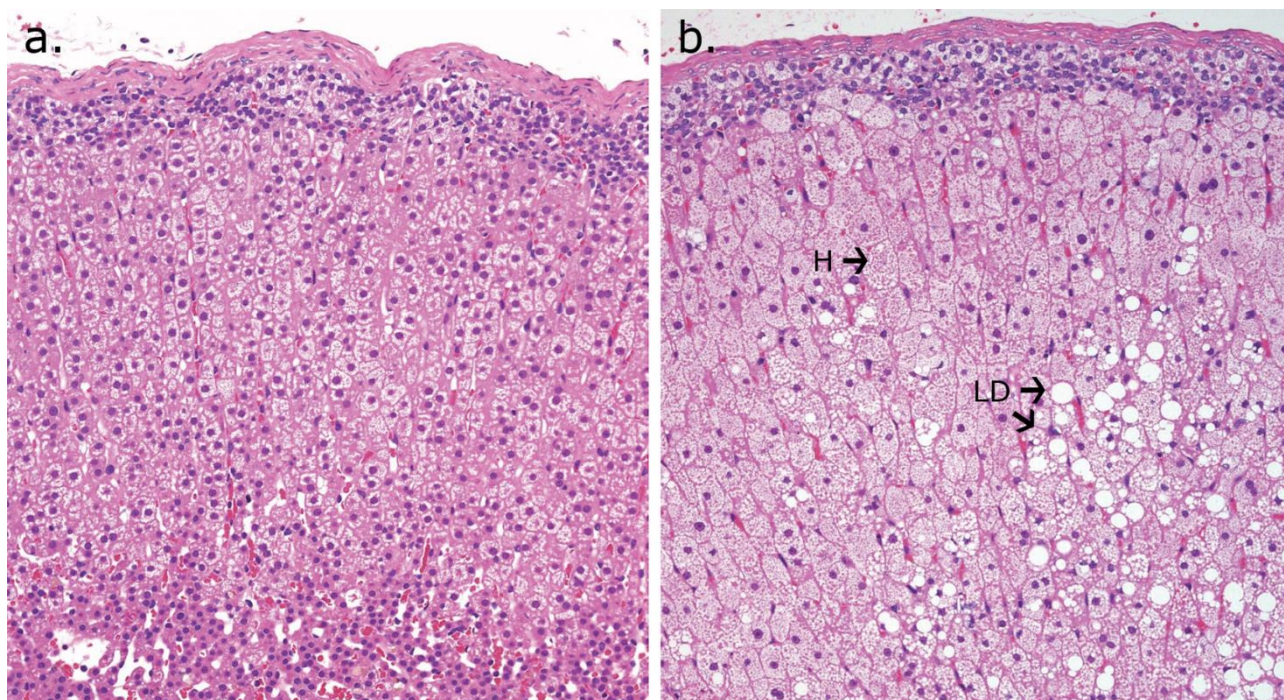
a. Adrenal gland, female (Animal No. 101), 0 dose: Normal.

b. Adrenal gland, female (Animal No. 161), 287 mg/kg bw/day: Severe diffuse hypertrophy and moderate diffuse vacuolation of cortical cells. LD: lipid droplets, H: hypertrophy of cortical cells, microvesiculated. The tissues shown in these photomicrographs were stained with haematoxylin and eosin and the magnification was ×160 for both plates.

In a mechanistic dietary study (Takeda, 2006), to further elucidate the effects on adrenal gland and ovary, male and female rats were exposed to doses of 0, 7.44 and 378 mg/kg bw/day for males and 0, 7.59 and 347 mg/kg bw/ day for females, for up to 28 days. Cyflumetofen caused enlarged and discoloured adrenals with increased weights. An increase in total cholesterol concentration, predominantly cholesteryl esters, in adrenals correlated with vacuolation by lipid deposition within the adrenocortical and ovarian interstitial cells at the high dose in both sexes. Furthermore, there was an inhibitory effect on HSL RNA expression (-21 to -31% relative to controls for males and females respectively), which is involved in cholesterol catabolism and therefore may be expected to result in lipid deposition- at the high dose in both sexes (table 121, CLH report). In addition, CYP11A1 (cholesterol side-chain cleavage enzyme) expression was slightly enhanced in both sexes at the high dose, probably due to the elevated supply of lipids. No effects were seen on **basal ACTH** or **corticosterone** levels in serum for either sex, the available data indicating that adrenal function was not affected. Unfortunately, there was no acute ACTH challenge performed to investigate if the corticosterone response in treated animals was lower than expected and so give some further indication if there was any organ functional deficiency arising from the disturbance of lipid metabolism in the adrenals.

In conclusion, the utilisation of cholesterol and the process of steroidogenesis may be impacted at cyflumetofen doses above 7.44 mg/kg bw/day, leading to the formation of lipid droplets in adrenals and ovarian cells. The effects seen in all repeated dose studies were vacuolation in adrenals and ovary, increased adrenal and liver weight and hypertrophy of hepatocytes.

Relevance of the effects for classification

The vacuolation of interstitial ovary cells was not accompanied by any additional histopathological change or functional disturbance of the ovary in studies with prolonged exposure or in reproductive toxicity studies.

The increase in liver weight and hypertrophy of hepatocytes was not accompanied by any further functional changes in liver, e.g. no relevant changes in clinical biochemistry were noted.

Observed effects in adrenal, ovary and liver are not considered to be toxicological significant because they did not result in organ damage, necrosis, fibrosis or granuloma formation, organ dysfunction or cell death, even with longer exposure times (chronic exposure duration).

In conclusion, the vacuolation and hypertrophy of the adrenal cortex *per se* is considered to be treatment related, but not a severe adverse effect that can result in functional impairment. There was no indication of dysfunction of the adrenal glands even after lifetime exposure. Similarly, effects in gonadal tissues resulted in **no** impairments to fertility in males or females.

The main target of cyflumetofen after repeated exposure is the adrenal gland (and steroidogenic tissues in general) as observed in rat, mouse and dog. The effect triggering the lowest endpoint was vacuolation of the adrenal cortical cells, which was observed in sub-acute, semi chronic and chronic toxicity studies. In the chronic studies the LOAEL for vacuolation of

adrenal cortical cells was similar to the LOAEL observed in semi chronic studies, indicating no increase of the effect after prolonged exposure. When considering all the studies, significant or severe toxicity related to the vacuolation of the adrenal cortical cells was **not** a feature for any of the tested species. There was no indication of functional disturbance in all of the available toxicity data on cyflumetofen. Morphological changes could be profound at very high doses (e.g. mouse adrenals) but without apparent toxicity. There was no indication of significant impacts on animal health in any of the toxicity studies.

Based on the study results above, it can be concluded that the cyflumetofen-induced vacuolation of adrenal cortical cells, of the ovary cells and increased liver weight, is not related to significant or severe toxicity. Therefore, the effects observed in repeated dose toxicity studies are considered by RAC **not to warrant classification of STOT RE**.

4.9 Germ cell mutagenicity (Mutagenicity)

Table 56 Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Results	Remarks	Reference
Ames test (OECD 471) 21-5000 µg/plate with and without S9	negative	Indicator cells: S. typh.TA 98, TA 100, TA 1535, TA 1537 E.coli WP2uvrA	[a]Matsumoto, 2001
chromosome aberration (OECD 473) Short-term treatment: 6.25-50 µg/ml without S9, 25-200 µg/ml with S9. Continuous (24 h) treatment: 3.75-30 µg/ml with and without S9.	negative	Chinese hamster lung (CHL) cells	[a]Matsumoto, 2003a
chromosome aberration assay (OECD 473) Short-term treatment: 0-80 µg/ml without S9, 0-320 µg/ml with S9. Continuous (18 h) treatment: 0 - 40 µg/ml without S9.	negative	V79 cells from Chinese Hamster	Schulz M., Landsiedel R. 2011
gene mutation (OECD 476) 20-90 µg/ml without S9, 10-140 µg/ml with S9.	mutagenic	mouse lymphoma cells L5178Y (TK)	[a]Verspeek-Rip, 2007
Micronucleus assay *in vivo* (OECD 474) 0, 500, 1000 and 2000 mg/kg bw	negative	Mouse bone marrow	[a]Matsumoto, 2003b

UDS *in vivo* (OECD 486) 0, 1000 and 2000 mg/kg bw	negative	Rat hepatocytes	[a]Buskens, 2007

[a] As summarised in revised DAR_2011_vol3 B6 (September 2011)

4.9.1   Non-human information

4.9.1.1 In vitro data

Reverse mutation assay (Matsumoto, 2001)

Reference	:	Matsumoto, K., 2001	vehicle :	dimethyl sulphoxide (DMSO)
Type of study	:	Ames test, preincubation method	GLP statement :	yes
year of execution	:	2001	guideline :	in accordance with OECD 471
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	acceptability :	acceptable
test system	:	*Salmonella typhimurium* and *Escherichia coli*	**Result** :	not mutagenic

Cyflumetofen was tested in the *Salmonella typhimurium* reverse mutation assay with four histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and in the *Escherichia coli* reverse mutation assay with a tryptophan-requiring strain of *Escherichia coli* (WP2uvrA). The test was performed in two independent experiments in the presence and absence of S9-mix (rat liver S9-mix induced by a combination of phenobarbital and ß-naphthoflavone). The test substance was dissolved in dimethyl sulfoxide. A preliminary cytotoxicity test was performed with the following dose range: 19.5, 78.1, 313, 1250 and 5000 µg/plate (a single plate per dose). Based on these results cyflumetofen was tested in the first experiment at concentrations of 19.5, 78.1, 313, 1250 and 5000 µg/plate and in the second experiment at concentrations of 20.6, 61.7, 185, 556, 1667 and 5000 µg/plate. In both experiments the doses were tested in triplicate.

The negative (vehicle) control used was dimethyl sulphoxide. The positive controls were 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), sodium azide (NaN$_3$) 9-aminoacridine hydrochloride (9-AA) (all without S9), and 2-aminoanthracene (2-AA) (with S9).

In the preliminary cytotoxicity assay cytotoxicity was not observed in any strain at any dose level. Precipitation was observed at the dose of 5000 µg/plate without metabolic activation.

The results of the main tests are summarised in **Table 57** and **Table 58**. A two-fold or more increase above the solvent control in the mean number of revertant colonies was not observed in the test substance group either in the absence or presence of a metabolic activation system. Precipitation was observed at the dose of 5000 µg/plate in the test substance group without a metabolic activation. Cytotoxicity was not observed in any strain at any dose level.

All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies, both with and without metabolic activation.

Table 57 Experiment 1, reverse mutation assay

With (+) or Without (-) S9-mix	Test substance concentration (µg/plate)	Number of colonies/plate [a]				
		Base-pair substitution type			Frame shift type	
		TA100	TA1535	WP2uvrA	TA98	TA1537
-S9 mix	Solvent control	113	6	21	18	5

	20.6	125	8	18	14	3
	61.7	119	5	26	14	6
	185	105	6	22	12	7
	556	126	6	19	13	4
	1667	107	6	21	17	6
	5000	100 *	10 *	20 *	10 *	5 *
+S9 mix	Solvent control	109	9	23	26	12
	20.6	140	7	30	28	14
	61.7	136	9	28	23	12
	185	135	7	27	32	8
	556	116	10	24	32	8
	1667	125	7	19	27	14
	5000	124	10	23	27	13
Positive control not requiring S9 mix	Name	AF-2	NaN3	AF-2	AF-2	9AA
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	517	599	247	510	532
Positive control requiring S9 mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	10	0.5	2
	Number of colonies/plate	907	162	231	250	57

a The average number of revertant colonies from three plates
) Precipitate

Table 58 Experiment 2, reverse mutation assay

With (+) or Without (-) S9-mix	Test substance concentration (μg/plate)	Number of colonies/plate [a]				
		Base-pair substitution type			Frame shift type	
		TA100	TA1535	WP$_2$uvrA	TA98	TA1537
-S9 mix	Solvent control	115	5	17	17	5
	156	120	4	23	16	5
	313	123	7	18	13	7
	625	108	8	21	18	4
	1250	108	7	24	13	3
	2500	105	9	19	16	4
	5000	104 *	5 *	16 *	15 *	5 *
+S9 mix	Solvent control	108	8	23	23	12
	156	131	6	19	21	13
	313	116	6	26	27	10
	625	133	8	24	27	10
	1250	126	5	23	29	8
	2500	116	10	19	26	14
	5000	129	5	21	31	8
Positive control not requiring S9 mix	Name	AF-2	NaN$_3$	AF-2	AF-2	9AA
	Concentration (μg/plate)	0.01	0.5	0.01	0.1	80
	Number of colonies/plate	473	613	253	587	531
Positive control requiring S9 mix	Name	2AA	2AA	2AA	2AA	2AA
	Concentration (μg/plate)	1	2	10	0.5	2
	Number of colonies/plate	957	162	223	274	83

a The average number of revertant colonies from three plates
) Precipitate

In this well performed test at dose levels up to and including 5000 µg/plate, the substance cyflumetofen (purity 97.67%) did not induce point mutations either in presence or absence of metabolic activation.

In vitro cytogenetic assay (Matsumoto, 2003a)

Reference	:	Matsumoto, K., 2003a	vehicle	:	dimethyl sulphoxide (DMSO)
Type of study	:	mammalian cell cytogenic assay (chromosome aberration)	GLP statement	:	Yes
year of execution	:	2003	guideline	:	in accordance with OECD 473
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	acceptability	:	Acceptable
test system	:	Chinese hamster lung cells	**Result**	:	not clastogenic

Cyflumetofen was tested in the *in vitro* cytogenetic test using cultured Chinese hamster lung cells (CHL). The test was performed in two independent experiments without and with a metabolic activation system (rat liver S9-mix induced by a combination of phenobarbital and ß-naphthoflavone). The test substance was dissolved in dimethyl sulfoxide.

A preliminary cytotoxicity test was performed with a dose range from 14 to 3580 µg/ml. In the short-term treatment, the test substance was treated at a concentration of 6.25, 12.5, 25 or 50 µg/ml for 6 hours without metabolic activation and at a concentration of 25, 50, 100 or 200 µg/ml for 6 hours with metabolic activation. Eighteen hours after the termination of the treatment, chromosome preparations were made. In the continuous treatment, the test substance was treated without metabolic activation at a concentration of 3.75, 7.5, 15 or 30 µg/ml for 24 and 48 hours.

Duplicate cultures were used for each concentration. 100 cells were examined per replicate culture (200 per dose) and were scored for structural aberrations and for numerical aberrations (polyploidy).

The negative (vehicle) control used was dimethyl sulphoxide. The positive controls were mitomycin C (MMC) without S9 and benzo(a)pyrene (B(a)P) with S9.

Nine doses ranging from 14 to 3580 µg/ml with and without S9 mix were evaluated. Precipitation was observed at 244 µg/ml and above both with and without metabolic activation. Over 50% cell growth inhibition was observed at the concentrations of 55.9 µg/ml and above without metabolic activation and at 244 µg/ml and more with metabolic activation. Over 50% cell growth inhibition was observed at the concentrations of 28 µg/ml and more both in the 24- and 48-hour treatment.

The results of the main tests are summarised in **Table 59 Short-term treatment process, without S-9 mix, 6 houra**, Table 59 to Table 62. In the 6 h exposure time, cyflumetofen did not induce a statistically significant or biologically relevant increase in the number of cells with chromosome aberrations, both in the absence and presence of a metabolic activation system. In the 24 and 48 h confirmatory trials again no statistically significant or biologically relevant increase in the number of cells with chromosome aberrations and/or polyploidy was observed.

The positive control chemicals (MMC-C and B(a)P) both produced statistically significant increases in the frequency of aberrant cells. It was therefore concluded that the test conditions were adequate and that the metabolic activation system (S9-mix) functioned properly.

Table 59 Short-term treatment process, without S-9 mix, 6 hour[a]

Treatment µg/ml	No. of Metaphases scored	Relative Cell Growth (%)	No. of Polyploid cells	No. of cells with structural chromosome aberrations								
				Gap	Chromatid type		Chromosome type		Frag-men-tation	Others	Total	
				g	ctb	cte	csb	cse			+g	-g
Solvent Control (DMSO)	100	100	2	0	2	0	0	1	0	0	3	3
	100	100	0	1	0	1	0	0	0	0	2	1
	Total (mean)	(100)	2 (1.0)	1 (0.5)	2 (1.0)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	0 (0)	5 (2.5)	4 (2.0)
6.25	100	95	0	0	0	0	0	0	0	0	0	0
	100	98	0	0	0	0	0	0	0	0	0	0
	Total (mean)	(97)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
12.5	100	89	0	0	0	0	0	0	0	0	0	0
	100	92	0	2	1	1	0	0	0	0	3	2
	Total (mean)	(91)	0 (0)	2 (1.0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
25	100	71	6	1	1	0	1	1	0	0	2	2
	100	69	2	2	0	1	0	0	0	0	3	1
	Total (mean)	(70)	8 (4.0)	3 (1.5)	1 (0.5)	1 (0.5)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	5 (2.5)	3 (1.5)
50	81	39	1	2	1	0	0	0	0	0	3	1
	91	38	0	1	0	0	0	0	0	0	1	0
	Total (mean)	(39)	1 (0.6)	3 (1.7)	1 (0.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.3)	1 (0.6)
Positive Control (MMC)	100	99	0	2	9	22	3	1	0	0	28	26
	100	100	1	8	16	27	4	0	0	0	42	40
	Total (mean)	(100)	1 (0.5)	10 (5.0)	25 (12.5)	49 (24.5)	7 (3.5)	1 (0.5)	0 (0)	0 (0)	70 (35.0)	66 (33.0) ***

Abbreviations: ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse chromosome exchange;
+g, including gaps; -g, excluding gaps; DMSO, dimethyl sulfoxide; MMC, mitomycin C
[a] CHL cells treated with the test substance for 6 hours in the absence of S9-mix and then cultured in fresh medium for further 18 hours
***) Significantly different from the solvent control at p <0.001

Table 60 Short-term treatment process, with S-9 mix, 6 hour[a]

Treatment µg/ml	No. of Metaphases scored	Relative Cell Growth (%)	No. of Polyploid cells	No. of cells with structural chromosome aberrations								
				Gap	Chromatid type		Chromosome type		Frag-men-tation	Others	Total	
				g	ctb	cte	csb	cse			+g	-g
Solvent Control (DMSO)	100	100	0	2	0	0	1	0	0	0	3	2
	100	100	0	0	1	0	0	0	0	0	0	0
	Total (mean)	(100)	0 (0)	2 (1.0)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
25	100	96	0	0	0	0	0	0	0	0	0	0
	100	98	1	0	0	1	0	0	0	0	1	1
	Total (mean)	(96)	1 (0.5)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.5)	1 (0.5)
50	100	92	1	0	0	2	0	0	0	0	2	2
	100	92	0	0	0	2	0	0	0	0	2	2
	Total (mean)	(92)	1 (0.5)	0 (0)	0 (0)	4 (2.0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.0)	4 (2.0)
100	100	78	0	1	0	1	0	0	0	0	2	1
	100	78	0	1	1	0	0	0	0	0	2	1
	Total (mean)	(78)	0 (0)	2 (1.0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0)	4 (2.0)	2 (1.0)
200 [b]	100	24	0	0	0	1	0	1	0	0	2	2
	100	24	0	0	0	1	0	0	0	0	1	1
	Total (mean)	(24)	0 (0)	0 (0)	0 (0)	2 (1.0)	0 (0)	1 (0.5)	0 (0)	0 (0)	3 (1.5)	3 (1.5)
Positive Control (B(a)P)	100	63	0	10	10	42	0	1	0	0	48	45
	100	64	1	8	17	40	0	0	1	0	55	52
	Total (mean)	(64)	1 (0.5)	18 (9.0)	27 (13.5)	82 (41.0)	0 (0)	1 (0.5)	1 (0.5)	0 (0)	103 (51.5)	97 (48.5) ***

Abbreviations: ctb, chromatid break; cte, chromatid exchange; csb, chromosome break; cse chromosome exchange; +g, including gaps; -g, excluding gaps;
DMSO, dimethyl sulfoxide; B(a)P, benzo(a)pyrene
[a] CHL cells treated with the test substance for 6 hours in the presence of S9-mix and then cultured in fresh medium for further 18 hours.
[b] Precipitation was observed immediately after addition of the preparation of the test substance
***) Significantly different from the solvent control at p <0.001

Table 61 Continuous treatment process, without S-9 mix, 24 hour

Treatment µg/ml	No. of Metaphases scored	Relative Cell Growth (%)	No. of Polyploid cells	Gap	Chromatid type		Chromosome type		Frag-men-tation	Others	Total	
				g	ctb	cte	Csb	cse			+g	-g
Solvent Control (DMSO)	100	100	0	1	0	0	0	1	0	0	2	1
	100	100	1	0	0	0	0	0	0	0	0	0
	Total (mean)	(100)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	1 (0.5)	0 (0)	0 (0)	2 (1.0)	1 (0.5)
3.75	100	102	0	0	0	0	0	0	0	0	0	0
	100	96	1	0	0	1	1	0	0	0	2	2
	Total (mean)	(99)	1 (0.5)	0 (0)	0 (0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	2 (1.0)	2 (1.0)
7.5	100	93	0	1	0	0	0	0	0	0	1	0
	100	88	0	0	0	1	1	0	0	0	2	2
	Total (mean)	(91)	0 (0)	1 (0.5)	0 (0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
15	100	78	0	1	0	0	1	0	0	0	2	1
	100	75	0	0	0	0	0	1	0	0	1	1
	Total (mean)	(77)	0 (0)	1 (0.5)	0 (0)	0 (0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
30	95	36	0	2	2	0	0	0	0	0	4	2
	77	42	0	1	2	2	0	0	0	0	5	4
	Total (mean)	(39)	0 (0)	3 (1.7)	4 (2.3)	2 (1.2)	0 (0)	0 (0)	0 (0)	0 (0)	9 (5.2)	6 (3.5)
Positive Control (MMC)	100	106	0	5	16	41	3	0	0	0	52	50
	100	95	0	6	16	36	4	1	0	0	45	44
	Total (mean)	(101)	0 (0)	11 (5.5)	32 (16.0)	77 (38.5)	7 (3.5)	1 (0.5)	0 (0)	0 (0)	97 (48.5)	94 (47.0) ***

Abbreviations: ctb, chromatid break;   cte, chromatid exchange;   csb, chromosome break;   cse chromosome exchange; +g, including gaps; -g, excluding gaps;   DMSO, dimethyl sulfoxide;   MMC, mitomycin C
***) Significantly different from the solvent control at p <0.001

Table 62 Continuous treatment process, with S-9 mix, 24 hour

Treatment µg/ml	No. of Metaphases scored	Relative Cell Growth (%)	No. of Polyploid cells	Gap	Chromatid type		Chromosome type		Frag-men-tation	Others	Total	
				g	ctb	cte	csb	cse			+g	-g
Solvent Control (DMSO)	100	100	0	0	0	0	1	0	0	0	1	1
	100	100	0	0	0	1	0	0	0	0	1	1
	Total (mean)	(100)	0 (0)	0 (0)	0 (0)	1 (0.5)	1 (0.5)	0 (0)	0 (0)	0 (0)	2 (1.0)	2 (1.0)
3.75	100	101	0	0	0	1	0	0	0	0	1	1
	100	98	1	1	0	0	0	1	0	0	2	1
	Total (mean)	(100)	1 (0.5)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
7.5	100	93	0	0	0	0	0	0	0	0	0	0
	100	91	0	0	0	0	0	0	0	0	0	0
	Total (mean)	(92)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
15	100	82	0	1	0	1	0	1	0	0	3	2
	100	78	0	0	0	0	0	0	0	0	0	0
	Total (mean)	(80)	0 (0)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	1 (0.5)	0 (0)	0 (0)	3 (1.5)	2 (1.0)
30 a)	0	20	-	-	-	-	-	-	-	-	-	-
	0	25	-	-	-	-	-	-	-	-	-	-
	Total (mean)	(23)	-	-	-	-	-	-	-	-	-	-
Positive Control (MMC)	100	110	2	3	19	33	6	2	0	0	48	47
	100	106	0	2	13	31	11	5	0	0	45	44
	Total (mean)	(108)	2 (1.0)	5 (2.5)	32 (16.0)	64 (32.0)	17 (8.5)	7 (3.5)	0 (0)	0 (0)	93 (46.5)	91 (45.5) ***

Abbreviations: ctb, chromatid break;   cte, chromatid exchange;   csb, chromosome break;   cse chromosome exchange;+g, including gaps; -g, excluding gaps;   DMSO, dimethyl sulfoxide;   MMC, mitomycin C
ª No metaphase cells because of cytotoxicity
*** Significantly different from the solvent control at p <0.001

Cyflumetofen did not induce chromosome aberrations in Chinese hamster lung cells either in the absence or presence of a metabolic activation system.

In vitro cytogenetic assay (Schulz, Landsiedel, 2011)

Reference	:	Schulz and Lansiedel, 2011	vehicle	:	dimethyl sulphoxide (DMSO)
Type of study	:	mammalian cell cytogenic assay (chromosome aberration)	GLP statement	:	Yes
year of execution	:	2011	guideline	:	in accordance with OECD 473
test substance	:	OK-5101 (batch 01H1; purity 97.08%)	acceptability	:	Acceptable
test system	:	V79 cells	**Result**	:	not clastogenic

Cyflumetofen was assessed for its potential to induce structural chromosomal aberrations (clastogenic activity) and/or changes in the number of chromosomes (aneugenic activity) in V79 cells *in vitro* both in the absence and the presence of a metabolizing system.

Nine doses of the test material ranging from 2.50 to 80 μg/mL in the absence and 10.00 to 160 ug/mL in the presence of S9-mix were evaluated in a preliminary growth inhibition test. Severe cytotoxicity was observed without the S9 mix. Based on this preliminary growth inhibition test, the following doses were tested in the main test:

1st Experiment
- 4-hour exposure, 18-hour sampling time, without S9 mix
    o 0; 2.5; 5.0; 10.0; 20.0; 40.0; 80.0 μg/mL
- 4-hour exposure, 18-hour sampling time, with S9 mix
    o 0; 10.0; 20.0; 40.0; 80.0; 120.0; 160.0 μg/mL

2nd Experiment
- 4-hour exposure, 18-hour sampling time, without S9 mix
    o 0; 0.31; 0.63, 1.25; 2.5; 5.0; 10.0 μg/mL

3rd Experiment
- 18-hour exposure, 18-hour sampling time, without S9 mix
    o 0; 0.31; 0.63, 1.25; 2.5; 5.0; 10.0; 20.0, 40.0 μg/mL
- 18-hour exposure, 28-hour sampling time, without S9 mix
    o 0; 2.5; 5.0; 10.0; 20.0, 40.0 μg/mL
- 4-hour exposure, 28-hour sampling time, with S9 mix
    o 0; 20.0; 40.0; 80.0; 120.0; 160.0; 240.0; 320.0 μg/mL

A sample of 100 metaphases for each culture was analyzed for chromosomal aberrations, except for the positive control cultures where only 50 metaphases were scored due to clearly increased aberration rates.

The results of the main tests are summarised in **Table 63** and **Table 64**.

Table 63: Chromosome aberration test with Cyflumetofen; Without S9 mix

Exp.	Schedule Exposure/ preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Incl. gaps#	Excl. Gaps#	With exchanges	Polyploid cells [%]	Cell number [%]	Mitotic index [%]
2	4/18 hrs	Vehicle control1	-	-	4.5	1.5	0.0	1.0	100.0	100.0
		0.31 μg/mL	-	-	2.0	1.5	1.0	0.0	104.7	145.1
		0.63 μg/mL	-	-	1.5	1.5	0.5	0.5	64.8	87.3

Exp.	Schedule	Test groups	S9 mix	P	Incl. gaps#	Excl. Gaps#	With exchanges	Polyploid cells [%]	Cell number [%]	Mitotic index [%]
		1.25 µg/mL	-	-	1.5	1.5	1.0	0.0	49.5	94.8
		2.50 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	52.8	90.2
		5.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	61.1	n.s.
		10.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	35.5	n.s.
		Positive control2X	-	-	13.0s	13.0s	8.0s	0.0	n.t.	85.0
3	18/18 hrs	Vehicle control1	-	-	4.0	1.0	0.0	0.0	100.0	100.0
		0.31 µg/mL	-	-	n.d.	n.d.	n.d.	n.d.	99.8	n.d.
		0.63 µg/mL	-	-	n.d.	n.d.	n.d.	n.d.	104.4	n.d.
		1.25 µg/mL	-	-	n.d.	n.d.	n.d.	n.d.	94.3	n.d.
		2.50 µg/mL	-	-	4.5	2.0	1.0	0.0	88.7	156.6
		5.00 µg/mL	-	-	5.0	3.0	1.0	1.0	102.0	144.1
		10.00 µg/mL	-	-	3.5	2.5	2.0	0.0	85.0	104.4
		20.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	61.8	n.s.
		40.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	54.4	n.s.
		Positive control2X	-	-	32.0s	28.0s	16.0s	0.0	n.t.	113.2
3	18/28 hrs	Vehicle control1	-	-	6.5	2.5	1.0	0.0	100.0	100.0
		2.50 µg/mL	-	-	n.d.	n.d.	n.d.	n.d.	99.2	n.d.
		5.00 µg/mL	-	-	n.d.	n.d.	n.d.	n.d.	109.3	n.d.
		10.00 µg/mL	-	-	2.0	1.5	1.5	1.5	87.0	87.8
		20.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	78.1	6.8
		40.00 µg/mL	-	-	n.s.	n.s.	n.s.	n.s.	5.3	n.s.
		Positive control2X	-	-	39.0s	39.0s	19.0s	0.0	n.t.	76.8

P Precipitation occurred at the end of exposure period
* Relative values compared with the respective vehicle control
# Inclusive cells carrying exchanges
n.t. Not tested
n.s. Not scorable due to poor metaphase quality
n.d. Not determined
S Aberration frequency statistically significant higher than corresponding control values
1 DMSO 1% (v/v)
2 EMS 500 μg/mL
x Evaluation of a sample of 100 metaphase only due to strong clastogenicity

Table 64: Chromosome aberration test with Cyflumetofen; With S9 mix

Exp.	Schedule Exposure/ preparation period	Test groups	S9 mix	P	Genotoxicity				Cytotoxicity*	
					Incl. gaps#	Excl. Gaps#	With exchanges	Polyploid cells [%]	Cell number [%]	Mitotic index [%]
1	4/18 hrs	Vehicle control1	+	-	2.5	**1.0**	0.5	0.5	100.0	100.0
		10.0 µg/mL	+	-	n.d.	**n.d.**	n.d.	n.d.	101.7	n.d.
		20.0 µg/mL	+	-	n.d.	**n.d.**	n.d.	n.d.	99.0	n.d.
		40.0 µg/mL	+	-	7.5	**4.0**	0.0	0.0	91.6	107.6
		80.0 µg/mL	+	-	3.5	**1.5**	0.0	0.5	71.9	76.3
		120.0 µg/mL	+	+	7.5	**4.5**	3.0	0.0	66.2	61.6
		160.0 µg/mL	+	+	n.s.	**n.s.**	n.s.	n.s.	34.8	11.2

		Positive control2X	+	-	24.0s	**22.0s**	13.0s	0.0	n.t.	80.4
3	4/28 hrs	Vehicle control1	+	-	4.5	**2.0**	1.0	0.0	100.0	100.0
		20.0 µg/mL	+	-	n.d.	**n.d.**	n.d.	n.d.	94.3	n.d.
		40.0 µg/mL	+	-	7.0	**2.5**	0.5	0.5	118.0	77.9
		80.0 µg/mL	+	-	8.0	**5.0**	4.0	0.0	128.3	86.3
		120.0 µg/mL	+	+	2.0	**1.0**	0.0	0.0	105.7	99.2
		160.0 µg/mL	+	+	n.s.	**n.s.**	n.s.	n.s.	150.4	n.s.
		240.0 µg/mL	+	+	n.s.	**n.s.**	n.s.	n.s.	153.7	n.s.
		320.0 µg/mL	+	+	n.s.	**n.s.**	n.s.	n.s.	163.1	n.s.
		Positive control2X	+	-	27.0s	**23.0s**	5.0s	2.0	n.t.	104.2

P Precipitation occured at the end of exposure period
* Relative values compared with the respective vehicle control
# Inclusive cells carrying exchanges
n.t. Not tested
n.s. Not scorable due to poor metaphase quality and/or strong cytotoxicity
n.d. Not determined
S Aberration frequency statistically significant higher than corresponding control values
1 DMSO 1% (v/v)
2 CPP 0.5 µg/mL
x Evaluation of a sample of 100 metaphase only due to strong clastogenicity

Under the experimental conditions described, cyflumetofen is considered not to have a chromosome-damaging (clastogenic) effect under in vitro conditions in V79 cells in the absence and the presence of metabolic activation.

In vitro gene mutation test (Verspeek-Rip, 2007)

Reference	:	Verspeek-Rip, C.M., 2007	vehicle	:	dimethyl sulphoxide (DMSO)
Type of study	:	mammalian cell gene mutation test	GLP statement	:	yes
year of execution	:	2007	guideline	:	in accordance with OECD 476
test substance	:	OK-5101 (batch 01H1; purity 98.4%)	acceptability	:	acceptable
test system	:	Mouse lymphoma cells L5178Y	**Result**	:	mutagenic

Cyflumetofen was tested in the *in vitro* mammalian cell gene mutation test using L5178Y mouse lymphoma cells. The test was performed without and with a metabolic activation system (rat liver S9-mix induced by a combination of phenobarbital and ß-naphthoflavone).
In the range finding test cultures were exposed to a range of doses from 1 to 333 µg/ml for 3 hours in the absence and presence of S9-mix and then subcultured in fresh medium twice for 24 hours. After each subculture cells were counted.
In the mutagenicity test, concentrations ranging from 0.5 to 90 µg/ml without S9 were tested and concentrations from 1 to 250 µg/ml with S9. Cells were exposed for 3 hours and incubated for 48 hours. Cells were separated, counted and cloning efficiency and mutation frequency were determined. Precipitation of cyflumetofen was observed at 90 µg/ml and higher. The concentrations used for determination of mutation frequency were 20-90 µg/ml without S9-mix and 10-140 µg/ml with S9-mix.
The negative (vehicle) control used was dimethyl sulphoxide. The positive controls were methyl methane sulfonate (MMS) without S9 and cyclophosphamide (CP) with S9.

In a range finding test, in the absence of S9-mix, no toxicity in the relative suspension growth was observed up to concentrations of 33 µg/ml compared to the relative suspension growth of the solvent control. Hardly any or no cell survival was observed at test substance concentrations of 100 and 333 µg/ml. In the presence of S9-mix, the relative suspension growth was 31% at the test substance concentration of 100 μg/ml compared to the relative suspension growth of the solvent control. Hardly any cell survival was observed at the test substance concentration of 333 μg/ml.

At concentrations of 90 µg/ml and higher cyflumetofen precipitated in the exposure medium.

The results of the main study are summarised in Table 65.

Table 65 Selection data and cloning efficiency

dose (µg/ml)	mutant colonies											total number of mutants			cloning efficiency (at day 2)				mutation frequency per 10^6 survivors		
	number of wells with mutants per selection plate [1]														n°. of empty wells per cloning plate		total number of empty wells	CE x 100%			
	1		2		3		4		5			s	l	s+l	1	2			total	small	large
	s	l	s	l	s	l	s	l	s	l											
*without metabolic activation*																				
SC1	7	8	7	4	13	6	9	12	10	6		46	36	82	33	31	64	110	85	46	35
SC2	6	8	8	6	6	7	7	6	7	7		34	34	68	41	39	80	88	87	42	42
20	4	2	12	4	6	6	9	8	6	7		37	27	64	44	37	81	86	83	46	34
30	9	6	9	6	17	6	7	4	12	5		54	27	81	29	36	65	108	85	55	27
40	8	6	6	5	9	5	14	4	14	7		51	27	78	32	33	65	108	82	52	27
50	12	6	9	7	11	8	17	13	13	12		62	46	108	32	30	62	113	113	61	45
60	14	3	13	9	14	12	11	7	18	11		70	42	112	30	35	65	108	123	73	42
70	17	12	14	11	15	11	19	14	20	12		85	60	145	34	32	66	107	168	91	63
80	24	7	33	5	15	8	16	14	26	11		114	45	159	44	37	81	86	233	157	57
90 [2]	33	9	22	7	31	4	27	14	32	10		145	44	189	38	40	78	90	278	200	53
MMS	19	6	19	7	21	8	14	7	27	9		270	92	362	45	50	95	70	673	469	143
	32	8	41	11	42	14	27	9	28	13											
*with metabolic activation*																				
	s	l	s	l	s	l	s	l	s	l		s	l	s+l							
SC1	16	9	17	8	11	12	11	2	11	10		66	41	107	28	31	59	118	107	63	38
SC2	6	4	15	8	8	12	5	8	6	8		40	40	80	29	31	60	116	78	37	37
10	16	6	8	4	9	4	6	3	14	7		53	24	77	34	28	62	113	77	52	23
20	11	5	10	12	12	15	13	12	10	8		56	52	108	35	28	63	111	114	56	51
40	13	4	8	8	10	13	8	9	8	6		47	40	87	25	28	53	129	78	40	34
60	6	7	14	9	12	7	10	6	9	5		51	34	85	29	26	55	125	78	45	29
80	14	6	8	6	6	11	10	3	12	7		50	33	83	35	30	65	108	88	51	33
100 [2]	11	6	10	9	12	14	9	11	16	7		58	47	105	29	33	62	113	109	57	46
120 [2]	24	9	30	15	36	8	23	7	29	11		142	50	192	34	29	63	111	229	157	49
140 [2]	43	18	49	17	43	18	46	15	50	18		231	86	317	40	34	74	95	566	344	104
CP	43	34	46	32	41	30	47	27	40	28		428	289	717	51	46	97	68	2012	865	525
	46	24	39	26	50	23	35	35	41	30											

s = small colonies
l = large colonies
[1] = solvent controls and treatment groups five plates with 2000 cells/well and the positive controls ten plates with 1000 cells/well
[2] = OK-5101 precipitated in the exposure medium

As the dose levels of 0.5 to 40 µg/ml showed no cytotoxicity in the absence of S9-mix, the dose levels 0.5, 1, 5 and 10 µg/ml were not used for mutation frequency measurement. In the presence of S9-mix, levels of 175 µg/ml and higher showed less than 10% cell viability and no dose level showed a cell survival between 10 and 20%. Therefore, this test was not used for determination of the mutation frequency and exposure with S9-mix was repeated. The concentrations used for determination of mutation frequency are 20-90 µg/ml without S9-mix and 10-140 µg/ml with S9-mix.

In the absence of S9-mix, cyflumetofen induced an increase in the mutation frequency at the TK locus up to 3.2 fold. Cyflumetofen showed up to 4.5- and 1.6-fold increases in the mutation frequency of the small and large colonies, respectively. In the presence of S9-mix, cyflumetofen induced an increase in the mutation frequency at the TK locus up to 6.1-fold. Cyflumetofen showed up to 6.9- and 2.8-fold increases in the mutation frequency of the small and large colonies, respectively. Although the increases were only observed at cytotoxic and/or precipitating dose levels, the increases in the mutation frequency were more than three-fold, were outside the historical control data range and in a dose-dependent manner.

Mutation frequencies in cultures treated with positive control chemicals were increased by 7.8-fold for MMS in the absence of S9-mix, and by 22-fold for CP in the presence of S9-mix. It was therefore concluded that the test conditions were adequate and that the metabolic activation system (S9-mix) functioned properly.

Cyflumetofen is mutagenic in the mouse lymphoma L5178Y test system under the experimental conditions described. As the increase in small colonies was larger than the increase in large colonies, cyflumetofen may have the potential to induce mainly chromosomal aberrations.

4.9.1.2 In vivo data

In vivo micronucleus test (Matsumoto, 2003b)

Reference	:	Matsumoto, K., 2003b	vehicle	:	0.5% carboxy-methylcellulose Na (CMC.Na)
Type of study	:	bone marrow micronucleus test	GLP statement	:	yes
year of execution	:	2001	guideline	:	in accordance with OECD 474
test substance	:	OK-5101 (batch 01D1; purity 97.67%)	acceptability	:	acceptable
test system	:	Mouse, ICR (Crj: CD-1) 5 males/dose	**Result**	:	not genotoxic

In a micronucleus test, mice (5 males/dose) were orally exposed to cyflumetofen (purity 97.67%) with a 24 hour interval for 2 days. Bone marrow smears were obtained at 24 hours after the second administration. The frequency of micronucleated polychromatic erythrocytes was calculated as the ratio of polychromatic erythrocytes (PCEs) with micronuclei among 2000 polychromatic erythrocytes. The ratio of polychromatic erythrocytes to normochromatic erythrocytes was calculated as the percentage polychromatic erythrocytes in 1000 erythrocytes. The vehicle was 0.5% CMC, the positive control was mitomicin C. Dose levels were 0, 500, 1000 and 2000 mg/kg bw.

No animals died at any dose level of cyflumetofen till 24 hours after the second administration. Furthermore, no clinical signs were observed at any dose level.

There were no statistically significant increases in the frequency of micronucleated polychromatic erythrocytes at any dose level of cyflumetofen. In the positive control group dosed with mytomicin C, on the other hand, the frequency of micronucleated polychromatic erythrocytes showed a highly significant increase.

From the ADME data it is clear that the test substances is able to reach the bone marrow after a single oral dose of 3 or 250 mg/kg bw to rats, and that elimination from bone marrow (half-life 14-30 hours) was slower than from plasma and other selected tissues. A toxicokinetic study in mice confirmed that cyflumetofen is rapidly absorbed and readily available in the plasma with peak levels after 0.5 hours in males.

Table 66 Summary of micronucleus results in male mice (Matsumoto, 2003b)

Sampling Time	Substance	Dose (mg/kg bw/d)	No. Of Mice	MNPCE/PCE (%)		PCE / (PCE +NCE) (%)	
				Mean	SD	Mean	SD
24 h post second administration	Vehicle (0.5% CMC-NA)	0	5	0.06	0.04	50.5	3.9
	Cyflumetofen	500 mg/kg	5	0.10	0.12	56.5	4.0
		1000 mg/kg	5	0.12	0.08	58.3	8.0
		2000 mg/kg	5	0.11	0.05	54.2	3.9
	Mitomycin C	10 mg/kg bw/d	5	4.29***	2.95	52.1	7.6

MNPCE: Micronucleated polychromatic erythrocytes
PCE: Polychromatic erythrocytes
NCE: Normochromatic erythrocytes
SD: Standard deviation
***: Significantly different from the concurrent vehicle control at p≤ 0.001

Cyflumetofen did not induce micronuclei in mouse bone marrow cells.

In vivo UDS test (Buskens, 2007)

Reference	:	Buskens, C.A.F, 2007		vehicle	:	Corn oil
Type of study	:	UDS		GLP statement	:	yes
year of execution	:	2007		guideline	:	in accordance with OECD 486
test substance	:	OK-5101 (batch 01H1; purity 98.4%)		acceptability	:	acceptable
test system	:	Rat, Wistar		**Result**	:	not genotoxic
		3 males/dose/sampling time				

An UDS test was performed, in which male rats were exposed by gavage to 1000 or 2000 mg/kg bw cyflumetofen (purity 98.4%). Hepatocytes were sampled 2-4 and 12-16 hours after administration. The vehicle was corn oil. The positive control was dimethylnitrosamine in water for 2-4 h treatment and 2-actylaminofluorene in corn oil for 12-16 h treatment. The test was in accordance with OECD 486.

The rats treated with cyflumetofen showed no abnormalities. Cell viability of hepatocytes from cyflumetofen treated rats was ≥76 and ≥74% for 2-4 and 12-16 hour sampling, respectively (73 and 80%, respectively, for vehicle control).

The nuclear grain count per slide and per animal, as well as the group average revealed no positive response in this assay at any of the dose levels. The percentage of cells in repair (repair taken as NNG ≥ 5), both per individual animal and per group, revealed no increase at any dose.

Results of positive and negative controls were within the expected range. The results are summarised in tables below.

Table 67   Results DNA repair assay, 2-4 hours sampling (Buskens, 2007)

Animal number	Dose mg/kg bw	NNG			% cells in repair	
		MEAN per animal	SD	group average	MEAN per animal	group average
14	Negative control 0	0.6	1.4		0	
16	Positive control 10	44.7	12.7		100	
18	Cyflumetofen 2000	0.3	1.4		0	

19	2000	0.5	1.3	0.5	0	0
20	2000	0.6	1.2		0	
21	1000	0.7	1.3		0	
22	1000	1.0	1.3	0.9	0	0
23	1000	0.8	1.3		0	

NNG   = net nuclear grain count; calculated for each cell by subtracting the cytoplasmic count from the nuclear count

MEAN   = mean of 100 cells (2 slides per animal)

group average   = average of 3 animals

SD   = standard deviation = σ_{n-1}

% cells in repair   : repair is taken as NNG $\geq$ 5

Table 68   Results DNA repair assay, 12-16 hours sampling (Buskens, 2007)

Animal number	Dose mg/kg bw	NNG			% cells in repair	
		MEAN per animal	SD	group average	MEAN per animal	group average
4	Negative control 0	0.7	1.3		0	
6	Positive control 50	31.9	11.7		100	
	Cyflumetofen					
8	2000	0.5	1.3		0	
9	2000	0.8	1.4	0.8	1	0.3
10	2000	1.1	1.3		0	
11	1000	0.9	1.1		0	
12	1000	0.7	1.2	0.7	0	0
13	1000	0.5	1.2		0	

NNG   = net nuclear grain count; calculated for each cell by subtracting the cytoplasmic count from the nuclear count

MEAN   = mean of 100 cells (2 slides per animal)

group average   = average of 3 animals

SD   = standard deviation = σ_{n-1}

% cells in repair   : repair is taken as NNG $\geq$ 5

4.9.2   Human information

No human data available.

4.9.3   Other relevant information

No other relevant data

4.9.4   Summary and discussion of mutagenicity

Cyflumetofen was tested for its genotoxic potential using a battery of in vitro (bacterial assay for gene mutation, clastogenicity in mammalian cells, and gene mutation in mammalian cells) and in vivo tests (mouse bone marrow micronucleus assay and rat hepatocyte DNA-repair assay).

Cyflumetofen was not mutagenic in the Ames test either in the absence or presence of a metabolic activation system in the strains tested and did not induce chromosome aberrations in Chinese hamster lung cells either in the absence or presence of a metabolic activation system. Cyflumetofen was mutagenic in the mouse lymphoma L5178Y test system. Cyflumetofen did not induce micronuclei in the bone marrow cells of ICR (Crj: CD) mice and is not genotoxic in the DNA-repair assay in hepatocytes from rats exposed in vivo.

Overall it is concluded that cyflumetofen does not have genotoxic potential in vivo.

4.9.5   Comparison with criteria

If there is evidence from in vitro or in vivo studies (or evidence in humans) that a substance (may) induce heritable mutations in humans, they should be classified for mutagenicity. Cyflumetofen does not fulfil these criteria.

4.9.6   Conclusions on classification and labelling

Cyflumetofen does not need to be classified for mutagenicity.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

In the CLH report, the DS summarised six genotoxicity studies which were compliant with the relevant OECD TG and GLP. The *in vitro* studies included an Ames test, mammalian gene mutation test and chromosome aberration tests. The *in vivo* studies included a micronucleus assay and an unscheduled DNA synthesis assay. Cyflumetofen was not mutagenic, clastogenic or genotoxic in any of the studies, with the exception of an *in vitro* mammalian gene mutation test conducted in mouse lymphoma L5178Y cells. Since the increase in small colonies in this study was larger than the increase in large colonies, the DS concluded that cyflumetofen may have the potential to induce chromosomal aberrations. There were no studies in germ cells.

Overall, the DS concluded that cyflumetofen does not have genotoxic potential *in vivo* and did not meet the criteria for classification for germ cell mutagenicity.

Comments received during public consultation

Two comments were received from industry addressing this endpoint. Both agreed with the DS proposal to not classify cyflumetofen for germ cell mutagenicity.

Assessment and comparison with the classification criteria

Cyflumetofen may be considered not to interact with the genetic material in a battery of genotoxicity assays for mutagenicity and clastogenicity.

In Vitro *Studies*

Exposure of *Salmonella typhimurium* and *Escherichia coli* tester strains to cyflumetofen at concentrations up to and including the limit concentration 5000 µg/plate in an Ames test did not produce an increased number of reversions, with or without S-9 mix metabolic activation (Matsumoto, 2001).

An *in vitro* assay for gene mutations in L5178YITK mouse lymphoma cells (Verspeek-Rip,

2007), with and without S9 mix, was <u>positive</u>. Cyflumetofen concentrations from 1 to 333 µg/mL used in a range-finding test revealed high cytotoxicity at 100 µg/mL, with reduction of relative suspension growth by 97% and 31% in the absence and presence of S9 mix, respectively. At 333 µg/mL, little cell survival was observed. In the mutagenicity test, the following concentrations were chosen for the gene mutagenicity assay: 0, 20, 30, 40, 50, 60, 70, 80 and 90 µg/mL for treatment without S9 mix and 0, 10, 20, 40, 60, 80, 100, 120 and 140 µg/mL for treatment with S9 mix. Mutation frequency was approximately 2-, 2.7- and 3.3-fold increased relative to controls at 70, 80 and 90 µg/mL, respectively, in the experiment without S9 mix and at a mean of 2.5- and 6.2-fold relative to controls at 120 and 140 µg/mL, respectively, in the experiment with S9 mix. Moreover, cyflumetofen revealed an up to 4.5- and 1.6-fold increase (in the absence of S9 mix) and an up to 6.9- and 2.8-fold increase (in the presence of S9 mix) in the mutation frequency of small and large colonies, respectively, which may suggest that cyflumetofen could mainly induce chromosomal aberration; this was, however, not confirmed by the *in vitro* chromosomal aberration studies in hamster cells and the *in vivo* micronucleus assay in mice.

The increase in mutation frequency was accompanied by cytotoxicity, reflected by a dose-related 64–91% reduction in relative total growth of cells in comparison to controls at 70 µg/mL and above in the experiment without S9 mix and a dose-related 56% and 90% reduction in relative total growth of cells in comparison to controls at 120 and 140 µg/mL in the experiment with S9 mix. Also of note, precipitation of cyflumetofen was observed at and above 90 µg/mL (without S9 mix) and 100 µg/mL (with S9 mix). A statistical analysis of the results was not performed in this study.

In conclusion, cyflumetofen was mutagenic in the mouse lymphoma L5178Y *in vitro* study. However, mutagenicity was observed only at cytotoxic and at saturated concentrations. In addition, if RAC assumes that the effects observed (an increase predominantly in the mutation frequency of small colonies relative to large colonies), suggest an induction of chromosomal aberration then these effects were not corroborated by the *in vitro* chromosomal aberration studies in hamster cells (Matsumoto, 2003a; Schulz, 2011) and the *in vivo* micronucleus assay in mice (Matsumoto, 2003b).

In Vivo *Studies*

Cyflumetofen was also negative in two *in vivo* genotoxicity assays. A bone marrow micronucleus in CD-1 mice did not reveal an increased frequency of micronucleated polychromatic erythrocytes following two oral doses of up to 2000 mg/kg bw. From the ADME data it is clear that the test substance is able to reach the bone marrow after a single oral dose of 3 or 250 mg/kg bw to rats, and that elimination from bone marrow (half-life 14-30 hours) was slower than from plasma and other selected tissues. DNA repair was not triggered in hepatocytes of Wistar rats treated orally with 1000 or 2000 mg cyflumetofen/kg bw.

Overall weight of evidence

RAC considers that the overall weight of evidence (table below) indicates cyflumetofen is not genotoxic.

Table: Summary of results from genotoxicity studies with cyflumetofen

End-point	Test system	Concentration	Batch no.; purity	Result	Reference
Reverse mutation (Ames)	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 *Escherichia coli* WP2 uvrA	20.6–5 000 µg/plate (±S9)	01D1; 97.67%	Negative[a]	Matsumoto (2001)
Mouse lymphoma TK	L5178Y cell line	10–140 µg/mL (±S9)[b]	01H1; 98.4%	Positive[b]	Verspeek-Rip (2007)
Chromosomal aberration	Chinese hamster lung cells	6.25–200 µg/mL (±S9)[c]	01D1; 97.67%	Negative	Matsumoto (2003a)
Chromosomal aberration	V79 Chinese hamster cells	0.31–320 µg/mL (±S9)[d]	01H1; 98.4%	Negative	Schulz (2011)
Micronucleated bone marrow cells	Male ICR (Crj: CD-1) mice	500, 1 000, 2 000 mg/kg bw in 5 males per dose	01D1; 97.67%	Negative	Matsumoto (2003b)
In vivo DNA repair assay (UDS)	Male Wistar (Han) rats	1 000 and 2 000 mg/kg bw in 3 males per dose	01H1; 98.4%	Negative	Buskens (2007)

[a] Precipitation of test substance without S9.
[b] More details are provided in the text above.
[c] In addition to a 6-hour exposure ±S9, a continuous treatment test was performed for 24 and 48 hours -S9.
[d] In addition to four tests with 4-hour exposure time ±S9, two tests with 18 hours of exposure -S9 were performed.

Comparison with the criteria

No human data are available for cyflumetofen, therefore a classification as Muta. 1A is not supported.

There are no data from *in vivo* heritable germ cell mutagenicity tests showing mutagenic effects in germ cells of humans therefore a classification as Muta. 1B is precluded. Cyflumetofen is negative in acceptable *in vitro* tests with the exception of the mouse lymphoma thymidine kinase (TK) gene mutation assay, and *in vivo* somatic cell mutagenicity guideline tests in mammals. However, the overall weight of evidence suggests no concern for mutagenicity.

RAC assumes that the effects observed in the *in vitro* studies (an increase predominantly in the mutation frequency of small colonies relative to large colonies), suggest an induction of chromosomal aberration however these effects were not corroborated by the *in vitro* chromosomal aberration studies in hamster cells (Matsumoto, 2003a; Schulz, 2011) and the *in vivo* micronucleus assay in mice (Matsumoto, 2003b).

Overall, RAC agrees with the DS that **classification is no warranted for germ cell mutagenicity**.

4.10 Carcinogenicity

Table 69 Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Carcinogenicity study (24 months) in rats, Fischer (F344/DuCrj) OECD 451 0, 150, 500 or 1500 mg/kg food equal to 0, 4.9, 16.5 and 49.5 mg/kg bw/d for males and 0, 6.1, 20.3 and 61.9 mg/kg bw/d	NOAEL: 16.5 mg/kg bw/day (m) NOAEL : 20.3 mg/kg bw/day (f) LOAEL: 49.5 mg/kg bw/day (m); LOAEL: 61.9 mg/kg bw/day (f)	Hypertrophy of the adrenal cortex and luminal dilatation of the gland in the uterine horn. No carcinogenicity at doses up to the highest level tested (1500 mg/kg food, equal to 49.5 mg/kg bw/day (m) or 61.9 mg/kg bw/day (f))	[a]Yoshida, 2004f
Carcinogenicity study (18 months) in mice, ICR (Crj:CD-1) OECD 451 0, 150, 500, 1500 or 5000 mg/kg food, equal to 0, 15.5, 54.3, 156 and 537 mg/kg bw/d for males and 0, 14.3, 48.1, 144 and 483 mg/kg bw/d for females	NOAEL: 156 mg/kg bw/day (m) NOAEL : 144 mg/kg bw/day (f) LOAEL: 537 mg/kg bw/day (m); LOAEL: 483 mg/kg bw/day (f)	Vacuolation of the adrenal cortex . No carcinogenicity at doses up to the highest level tested (5000 mg/kg food, equal to 537 mg/kg bw/day (m) or 483 mg/kg bw/day (f))	[a]Yoshida, 2004g
Carcinogenicity study (78 weeks) in mice, ICR (Crj: CD-1) (MTD) OECD 451, EPA 870.4200; JMAFF No 12 Nosan No 8147 0 or 10000 mg/kg food, equal to 0 and 1143 mg/kg bw/d for males and 0 and 1132 mg/kg bw/d for females	LOAEL: 1143 mg/kg bw/day (m); LOAEL: 1132 mg/kg bw/day (f)	No carcinogenicity; effects on adrenal weights (f), significant increases in the incidences of diffuse vacuolation of cortical cell and deposition of brown pigment in the cortico-medullary junction in the adrenal gland (m, f)	[b] Yoshida, 2013
Carcinogenicity study (104 weeks) in rats, F344/DuCrlCrlj (MTD) OECD 451, JMAFF No 12 Nosan No 8147, EPA 870.4200 0 or 6000 mg/kg food, equal to 0 and 220 mg/kg bw/d for males and 0 and 287 mg/kg bw/d for females	LOAEL: 220 mg/kg bw/day (m); LOAEL: 287 mg/kg bw/day (f)	Increased incidence of Leydig cell tumours. Decreased body weights, increased food consumption, changes in haematological parameters (f), changed organ weights, atrophy pancreas acinar focal cell (m), decreased hyperplasia interstitial cell and decreased atrophy of seminiferous tubule, histopathological findings adrenals	[b] Takahashi, 2013

[a] As summarised in revised DAR_2011_vol3 B6 (September 2011)
[b] Additional studies performed for US-EPA

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

104-week oral carcinogenicity study in rats (Yoshida, 2004f)

reference	:	Yoshida, T., 2004f	exposure	:	24 months, diet
type of study	:	24-months carcinogenicity study	doses	:	: 0, 150, 500, 1500 mg/kg food[1]
year of execution	:	2001-2004	vehicle	:	None
test substance	:	OK-5101 batch no: 01D1; purity 97.67%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 451
species	:	Rat, Fischer (F344/DuCrj)	acceptability	:	Acceptable
group size	:	50/sex/dose	**NOAEL**	:	16.5 mg/kg bw/d (M) 20.3 mg/kg bw/d (F)

[1] equal to 0, 4.9, 16.5 and 49.5 mg/kg bw/d for males and 0, 6.1, 20.3 and 61.9 mg/kg bw/d for females

In a 104 week carcinogenicity study rats were exposed to cyflumetofen (purity 97.67%) at dietary levels of 0, 150, 500 or 1500 mg/kg food. The dose levels were equal to 0, 4.9, 16.5 and 49.5 mg/kg bw/d for males and 0, 6.1, 20.3 and 61.9 mg/kg bw/d for females. The study was performed according to OECD 451. Toxicologically relevant effects were noted in rats treated with cyflumetofen at 1500 mg/kg food for 104 weeks (see Table 70).

No effect on mortality, clinical signs, body weight or food consumption was noted.

Decreased lymphocyte count showed no dose-relationship and was without correlated histopathological findings, and therefore considered to be not toxicologically relevant. The toxicological significance of the decreased eosinophil count is not clear (see Table 71). The increased absolute and relative pituitary weights at 150 mg/kg food in females and at 500 and 1500 mg/kg food in males was due to the presence of a large anterior adenoma compared to the control group in 1-2 animals. The decreased absolute and relative spleen weight at 1500 mg/kg food and the decreased absolute spleen weight at 500 mg/kg food in females were caused by a huge spleen weight in one female in the control group due to mononuclear cell leukaemia. Absolute and relative adrenal weight in females at 1500 mg/kg food was increased (109 and 118% of control). The decreased absolute and relative adrenal weights in males were caused by a huge adrenal weight in one male in the control group due to a complex pheochromocytoma. Evaluation of the adrenal weights in males after exclusion of this male revealed an increased absolute and relative weight at 500 (121 and 123% of control) and 1500 mg/kg food (116 and 115% of control), and in absence of a dose-response and since at 500 mg/kg food in males no increased incidence in histopathological changes in adrenals were noted, the observed increase in adrenal weight at 500 mg/kg food is not considered to be adverse. Organ weights are summarized in Table 72, Table 73 and Table 74.

At macroscopy the incidence of atrophy of the epididymides was slightly increased at 1500 mg/kg food in males killed at termination. A higher incidence of enlarged lymph nodes was noted at 1500 mg/kg food in males killed in extremis. The incidence of a mass in the testes was increased in all treated males without a dose-response relationship. In the absence of histopathological findings for any of the above effects, the macroscopic effects are considered to be not toxicologically relevant. Testicular masses were found at macroscopy. Slightly higher incidences were observed at the high dose, however without statistical significance and no dose-response was seen. As a histopathological counterpart, interstitial cell tumours were seen, however, also without statistical significance or a clear dose response (see Table 76). Although the incidence of interstitial cell tumours at the high dose level (46/50) was slightly above the historical control range (34/50 to 43/50, see Table 77), it is noted that the incidence in control males (of the study was already at the upper range of the historical control range. Almost all animals that survived until terminal kill had

interstitial cell tumours (41/42 in control and 40/41 in the high dose). Furthermore, as seen in the historical control range, interstitial cell tumours in tests are a common finding in rats, especially in the Fisher F344 strain.

Histopathology showed an increased incidence of diffuse hypertrophy of adrenal cortical cells in most males and females at 1500 mg/kg food. This finding correlates with the increased adrenal weight noted at this dose level. An increased incidence of luminal dilatation of the gland in the uterine horn was seen at 1500 mg/kg food in females killed at termination. The incidence of neoplastic lesions was similar for treated and control animals. Considering the incidence of anterior pituitary adenoma found in the historical controls, the incidence found in the 24-month study in rats is not considered treatment related (see Table 65 and 66).

Based on hypertrophy of the adrenal cortex and luminal dilatation of the gland in the uterine horn, the NOAEL is set at 500 mg/kg food (equal to 16.5 and 20.3 mg/kg bw/d for males and females, respectively). In this study cyflumetofen showed no carcinogenic potential.

Table 70 Summary table (Yoshida, 2004f)

Dose (mg/kg food)	0		150		500		1500		dr
	m	f	m	f	m	f	m	f	
Mortality (n=50)	8	7	10	8	8	9	9	5	
Clinical signs	no treatment-related findings								
Body weight	no treatment-related findings								
Food consumption	no treatment-related findings								
Haematology									
- lymphocyte count			dc				dc		
- eosinophil count							dc		
Organ weights									
- adrenals					ic[a,r]		ic[a]	i[a,r]	
Pathology									
macroscopy									
Terminal kill:									
- epididymis, atrophy	36/42		35/40		40/42		41/41		
Killed in extremis:									
- lymph node, enlarged	0/8	0/7	2/10	0/8	3/8	1/9	5/9	1/5	
- testis, mass	0/8		5/10		4/7		6/9		

Dose (mg/kg food)	0		150		500		1500		dr
	m	f	m	f	m	f	m	f	
microscopy									
neoplastic lesions									
Testes: interstitial cell tumour	43/50		42/49		43/48		46/50		
Pituitary: anterior adenoma	4/50	16/43	6/23	12/25	6/17	12/29	6/50	16/45	
non-neoplastic lesions									
Terminal kill:									
- adrenal, cortical cell hypertrophy	4/42	3/43	2/40	3/42	4/42	1/41	16/41	22/45	
- uterine horn, luminal dilatation, gland		6/43		7/42		9/41		14/45	
Killed in extremis:	no treatment-related findings								

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative

Table 71 Selected haematology findings in rats administered cyflumetofen for 2 years (group means) (Yoshida, 2004f)

Dose (ppm)	Lymphocyte (L) $10^3/\mu L$	(L) % of Control	Eosinophil (E) $10^3/\mu L$	(E) % of Control
MALE				
Control	3.75	100	0.12	100
150	3.09*	82	0.11	92
500	4.64	124	0.11	92
1500	2.97**	79	0.10*	83
FEMALE				
Control	3.14	100	0.08	100
150	3.20	102	0.07	88
500	2.51	80	0.07	88
1500	2.44	78	0.07	88

* $p \leq 0.05$; ** $p \leq 0.01$

Table 72 Selected organ weight findings in rats administered cyflumetofen for 2 years (group means) (Yoshida, 2004f)

Dose (ppm)	Parameter											
	Pituitary				Spleen				Adrenal			
	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl
MALE												
Control	13.7	100	0.0032	100	1069	100	0.25	100	72.8	100	0.017	100
150	13.5	99	0.0031	97	1028	96	0.24	96	57.2	79	0.013	76
500	37.8	276	0.0094*	294	973	91	0.23	92	67.4*	93	0.016*	94
1500	33.7	246	0.0076	238	1051	98	0.24	96	64.4	88	0.015	88

Dose (ppm)	Parameter											
	Pituitary				Spleen				Adrenal			
	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl
FEMALE												
Control	20.3	100	0.0072	100	1440	100	0.052	100	62.4	100	0.022	100
150	42.8	211	0.0168	233	970	67	0.36	69	63.0	101	0.024	109
500	16.6	82	0.0063	88	506*	35	0.19	37	62.8	101	0.024	109
1500	19.3	95	0.0072	100	500**	35	0.19*	37	68.1	109	0.026	118

* p $\leq$ 0.05; ** p $\leq$ 0.01

Table 73   Absolute and relative spleen weight females, recalculated excluding control animal no. 203. (Yoshida, 2004f)

mg/kg food	0 (unadjusted)	0 (adjusted)	150	500	1500
	Absolute spleen weight [mg]				
Mean	1440	655	970	506	500
SD	2486	159	983	99	92
N	10	9	10	10	10
	Relative spleen weight				
Mean	0.52	0.23	0.36	0.19	0.19
SD	0.91	0.05	0.34	0.04	0.04
N	10	9	10	10	10

Table 74 Adrenal weight after exclusion of one male of the control group   (Yoshida, 2004f)

Parameter		Dose group (ppm)			
		Male			
		0	150	500	1500
Adrenal	Absolute weight	100 (55.7±5.2 mg)	103 (57.2±3.7 mg)	↑121 (67.4±9.7 mg)	↑116 (64.4±9.0 mg)
	Relative weight	100 (0.013±0.002%)	100 (0.013±0.001%)	↑123 (0.016±0.003%)	115 (0.015±0.002%)

Figures show the ratio (%) to the control value (100%).   Mean ± SD are shown in parenthesis.
Dunnett's multiple comparison test: ↑, P≤0.01   ↑, P≤0.05.
Re-evaluated data after one male of the control group (No. 10) was excluded.

Table 75   Incidence of cortical cell vacuolation (Yoshida, 2004f)

Dose in mg-kg food	0		150		500		1500	
	m	f	m	f	m	f	m	f
Cortical cell vacuolation, focal								
Terminal kill, 104 weeks	11/42	17/43	1/40	11/42	9/42	19/41	10/41	18/45
Killed in extremis/found dead	1/8	1/7	1/10	3/8	1/8	0/9	2/9	0/5
All animals examined	12/50	18/50	8/50	14/50	10/50	19/50	12/50	18/50
Cortical cell vacuolation, diffuse								
Terminal kill, 104 weeks	0/42	-	1/40	-	3/42		4/41	-
Killed in extremis/found dead	3/8	1/7	2/10	0/8	0/8	5/9	0/9	1/5
All animals examined	3/50	1/50	3/50	0/50	3/50	5/50	4/50	1/50

a   examined on the animals that showed macroscopic lesions

-          not observed

Table 76   Incidence of testicular masses and interstitial cell tumours (Yoshida, 2004f)

Dose in mg-kg food	0		150		500		1500	
	m	f	m	f	m	f	m	f
Testes, Mass(es)								
Terminal kill, 104 weeks	38/42		36/40		35/42		39/41	
Killed in extremis/found dead	0/8		5/10		4/7		6/9	
All animals examined	38/50		41/50		39/49		45/50	
Testes, interstitial cell tumours								
Terminal kill, 104 weeks	41/42		37/39a		39/41a		40/41	
Killed in extremis/found dead	2/8		5/10		4/7		6/9	
All animals examined	43/50		42/49		43/48		46/50	

a   examined on the animals that showed macroscopic lesions

-          not observed

Table 77   Historical control data testicular interstitial cell tumours, F344 rats from IET carcinogenicity studies

	Study ID		A	B	C	D	E	F	G
	Year		1991	1993	1996	1996	1996	1997	2001
	total	%							
No. of animals	350		50	50	50	50	50	50	50
Testicular interstitial cell tumours									
Male	277	79.14	43	43	39	40	34	35	43
Female	-	-	-	-	-	-	-	-	-

Table 78   Incidence of anterior pituitary adenoma (Yoshida, 2004f)

Dose in mg-kg food	0		150		500		1500	
	m	f	m	f	m	f	m	f
Anterior pituitary adenoma								
Terminal kill, 104 weeks	3/42	16/43	4/13a	12/25a	6/9a	12/29a	5/41	16/45
Killed in extremis/found dead	1/8	2/7	2/10	3/8	0/8	2/9	1/9	3/5
All animals examined	4/50	18/50	6/23	15/33	6/17	14/38	6/50	19/50

a   examined on the animals that showed macroscopic lesions.

Table 79 Historical control data anterior pituitary adenoma, F344 rats from IET carcinogenicity studies

	Study ID		A	B	C	D	E	F	G
	Year		1991	1993	1996	1996	1996	1997	2001
	total	%							
No. of animals	350		50	50	50	50	50	50	50
Anterior pituitary adenoma									
Male	81	23.14	7	6	15	18	14	13	8
Female	153	43.71	17	19	22	26	25	28	16

18-month oral carcinogenicity study in mice (Yoshida, 2004g)

reference	:	Yoshida, T., 2004g	exposure	:	18 months, diet
type of study	:	18-months carcinogenicity study	doses	:	: 0, 150, 500, 1500 or 5000 mg/kg food[1]
year of execution	:	2002-2004	vehicle	:	None
test substance	:	OK-5101 batch no: 01D1; purity 97.67%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 451
species	:	Mouse, ICR (Crj: CD-1)	acceptability	:	Acceptable
group size	:	52/sex/dose	**NOAEL**	:	156 mg/kg bw/d (M) 144 mg/kg bw/d (F)

[1] equal to 0, 15.5, 54.3, 156 and 537 mg/kg bw/d for males and 0, 14.3, 48.1, 144 and 483 mg/kg bw/d for females

In an 18 month carcinogenicity study mice were exposed to cyflumetofen (purity 97.67%) at dietary levels of 0, 150, 500, 1500 or 5000 mg/kg food. The dose levels were equal to 0, 15.5, 54.3, 156 and 537 mg/kg bw/d for males and 0, 14.3, 48.1, 144 and 483 mg/kg bw/d for females. The study was performed according to OECD 451. Toxicologically relevant effects were noted in mice treated with cyflumetofen at 5000 mg/kg food for 78 weeks (see summary Table 80).

Mortality, clinical signs, body weight, food consumption and haematology were not affected by treatment. In males at 500 mg/kg food cumulative mortality was increased from about week 60 onwards. Since no effect at 1500 and 5000 mg/kg food was observed on mortality, this effect is not considered to be treatment-related.

Absolute adrenal weight was increased in males at 5000 mg/kg food (120% of control). This was caused by cortical cell hypertrophy in 2 males, however, no increased incidence of cortical cell hypertrophy was noted in males at 5000 mg/kg food (see Table 81). Increased absolute and relative spleen weight at 5000 mg/kg food in males (199 and 200% of control, respectively) was due to the presence of a malignant lymphoma in 2 males. Since at histopathology no increased incidence of malignant lymphoma was noted at 5000 mg/kg food the effect was considered to be not treatment-related.

In males killed *in extremis* an increased incidence of ascites in the abdominal cavity was noted. Since no increased incidence was seen in females and no histopathological confirmation was observed, the effect was considered to be not toxicologically relevant.

The incidence of neoplasms was similar for treated and control animals. Additional information on the incidence of systemic malignant lymphomas and spleen tumours, including historical control data are presented in Table 82 and Table 83. Based on the incidence of tumours found, it can be concluded that there is no increased incidence in systemic malignant lymphomas or spleen tumours.

An increased incidence of diffuse vacuolation of cortical cells (not hypertrophy) in the adrenals was noted at 5000 mg/kg food in both sexes at terminal kill. The incidence at 1500 mg/kg food is not considered increased when compared to controls for both males and females and is within the historical control range (see Table 84 and Table 85).

Based on vacuolation of the adrenal cortex, the NOAEL is set at 1500 mg/kg food (equal to 156 and 144 mg/kg bw/d for males and females, respectively). In this study cyflumetofen showed no carcinogenic potential.

Table 80 Summary table (Yoshida, 2004g)

Dose (mg/kg food)	0		150		500		1500		5000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality (n=52)	15	15	18	13	23	13	19	15	15	18	
Clinical signs	no treatment-related findings										
Body weight	no treatment-related findings										
Food consumption	no treatment-related findings										
Haematology	no treatment-related findings										
Organ weights - adrenals - spleen									i[a] i[a,r]		
Pathology											
macroscopy Terminal kill:	no treatment-related findings										
Killed in extremis: - abdominal cavity, ascites	0/15	2/15	1/18	2/13	0/23	2/13	0/19	4/15	4/15	0/18	
microscopy _neoplastic lesions_	no treatment-related findings										
non-neoplastic lesions Terminal kill: - adrenal, cortical cell vacuolation	1/37	2/37	1/34	3/39	1/29	2/39	2/33	5/37	8/37	18/34	
Killed in extremis:	no treatment-related findings										

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative

Table 81   Incidence of cortical cell hypertrophy (Yoshida, 2004g)

Dose in mg-kg food	0		150		500		1500		5000	
	m	f	m	f	m	f	m	f	m	f
Cortical cell hypertrophy, focal										
Terminal kill, 78 weeks	9/37	-	6/34	-	4/29	-	5/33	-	4/37	-
Killed in extremis/found dead	1/15	-	1/18	-	0/23	-	1/19	-	0/15	-
All animals examined	10/52	-	7/52	-	4/52	-	6/52	-	4/52	-
Cortical cell hypertrophy, diffuse										
Terminal kill, 78 weeks	0/37	-	2/34	-	1/29	-	0/33	-	2/37	-
Killed in extremis/found dead	2/15	-	5/18	-	3/23	-	1/19	-	3/15	-

All animals examined	2/52	-	7/52	-	4/52	-	1/52	-	5/52	-

a   examined on the animals that showed macroscopic lesions
-    not observed

Table 82   Incidence of neoplastic lesions in spleen and lymphatic system (Yoshida, 2004g)

Dose in mg-kg food	0		150		500		1500		5000	
	m	f	m	f	m	f	m	f	m	f
Systemic tumour: malignant lymphoma (lymphocytic)										
Terminal kill, 78 weeks	1/37	3/37	1/34	1/39	0/29	1/39	1/33	0/37	2/37	2/34
Killed in extremis/found dead	4/15	4/15	1/18	5/13	2/23	3/13	3/19	4/15	1/15	6/18
All animals examined	5/52	7/52	2/52	6/52	2/52	4/52	4/52	4/52	3/52	8/52
Systemic tumour: malignant lymphoma (mixed cell)										
Terminal kill, 78 weeks	0/37	0/37	0/34	1/39	0/39	0/39	0/33	1/37	1/33	0/34
Killed in extremis/found dead	0/15	1/15	0/18	1/13	0/23	0/13	0/19	1/15	0/15	0/18
All animals examined	0/52	1/52	0/52	2/52	0/52	0/52	0/52	2/52	1/52	0/52
Spleen: hemangiosarcoma										
Terminal kill, 78 weeks	0/37	0/37	1/3a	1/7a	0/3a	1/5a	1/4a	0/4a	0/37	0/34
Killed in extremis/found dead	1/15	0/15	0/18	0/13	0/23	0/13	1/19	1/15	0/15	0/18
All animals examined	1/52	0/52	1/21	1/20	0/26	1/18	2/23	1/19	0/52	0/52
Spleen: hemangioma										
Terminal kill, 78 weeks	0/37	0/37	0/3a	0/7a	0/3a	0/5a	0/4a	0/4a	0/37	0/34
Killed in extremis/found dead	0/15	0/15	0/18	0/13	0/23	0/13	1/19	0/15	0/15	0/18
All animals examined	0/52	0/52	0/21	0/20	0/26	0/18	1/23	0/19	0/52	0/52
Lymph node: malignant lymphoma (lymphocytic)										
Terminal kill, 78 weeks	0/37	-	0/34	-	0/29	-	0/33	-	0/37	-
Killed in extremis/found dead	0/15	-	0/18	-	0/23	-	1/19	-	0/15	-
All animals examined	0/52	-	0/20	-	0/24	-	1/20	-	0/52	-

a   examined on the animals that showed macroscopic lesions
-    not observed

Table 83 Historical control data malignant lymphoma and spleen hemangiosarcoma, ICR (Crj:CD-1) mice from IET carcinogenicity studies

	Study ID		A	B	C	D	E	F
	Year		1991	1993	1995	1996	1999	2001
	total	%						
No. of animals	310		52	52	50	52	52	52
Systemic tumour: malignant lymphoma								
Male	19	6.13	2	3	2	3	3	6
Female	49	15.81	14	7	6	9	5	8
Spleen: hemangiosarcoma								
Male	4	1.29	1	1	0	0	0	2
Female	2	0.65	0	0	0	0	0	2

Table 84 Incidence of cortical cell vacuolation (Yoshida, 2004g)

Dose in mg-kg food	0		150		500		1500		5000	
	m	F	m	f	m	f	m	f	m	f
Cortical cell vacuolation, focal										
Terminal kill, 78 weeks	-	-	-	-	-	-	-	-	-	-
Killed in extremis/found dead	-	-	-	-	-	-	-	-	-	-
All animals examined	-	-	-	-	-	-	-	-	-	-
Cortical cell vacuolation, diffuse										
Terminal kill, 78 weeks	1/37	2/37	1/34	3/39	1/29	2/39	2/33	5/37	8/37*	18/34**
Killed in extremis/found dead	0/15	5/15	2/18	1/13	1/23	2/13	1/19	4/15	0/15	8/18
All animals examined	1/52	7/52	3/52	4/52	2/52	4/52	3/52	9/52	8/52*	26/52**

a   examined on the animals that showed macroscopic lesions
-      not observed
*   significantly different from the control at 5% level of probability
**   significantly different from the control at 1% level of probability

Table 85 Historical control data adrenal gland: vacuolation, cortical cell, diffuse in [Crlj:CD1(ICR)] mice from IET carcinogenesis studies

Initiation of study	1996	1999	2001	2003	2003	2003	2004
male	1/52	0/52	2/52	1/52	3/52	2/52	4/52

female	8/52	5/52	5/52	4/52	5/52	9/52	4/52

78-week oral carcinogenicity study in mice (Yoshida, 2013)

reference	:	Yoshida, T., 2013	exposure	: 78-weeks, diet
type of study	:	78-week carcinogenicity study	doses	: 0, 10000 mg/kg food[1]
year of execution	:	2011-2013	vehicle	: None
test substance	:	OK-5101; purity 97.82%	GLP statement	: Yes
route	:	Oral	guideline	: According to OECD 451
species	:	Mouse, ICR (Crj: CD-1)	acceptability	: Acceptable
group size	:	52/sex/dose	**NOAEL**	: <1143 mg/kg bw/d (M)
				< 1132 mg/kg bw/d (F)

[1] Corresponding to 0, 1143 mg/kg bw/d for males and 0, 1132 mg/kg bw/d for females

In a 78 week carcinogenicity study mice were exposed to cyflumetofen (purity 97.82%) at dietary levels of 0 or 10000 mg/kg food. The dose levels were equal to 0 and 1143 mg/kg bw/d for males and 0 and 1132 mg/kg bw/d for females. The study was performed according to OECD 451. Toxicologically relevant effects were noted in mice treated with cyflumetofen at 10000 mg/kg food for 52 weeks (see Table 86 and Table 87).

There were no relevant changes in body weights and no significant change in mean food consumption in males and females. At 10,000 mg/kg food, females showed significant increases in the incidences of pale-coloured skin and pale-coloured eye/eyelid. In organ weight, females showed significant increases in absolute and relative weights of the adrenals. In histopathological examination, both males and females showed significant increases in the incidences of diffuse vacuolation of cortical cell and deposition of brown pigment in the cortico-medullary junction in the adrenal gland. Furthermore, females at 10,000 mg/kg food showed a significant increase in the incidence of increased extramedullary haematopoiesis in the spleen (all animals examined). The observed incidence at 10,000 mg/kg food was higher than in historical control data (2-5/52 in studies of the same laboratory from 2008-2012). However, the hematopoiesis could not be linked to neoplastic findings. Increased extramedullary haematopoiesis in the spleen might be caused by secondary effects to other organs and tissues; animals with increased extramedullary haematopoiesis in the spleen also showed e.g. cystitis in urinary bladder, dermatitis in skin, arteritis in thymus, hematoma in uterine horn, etc.

At 10,000 mg/kg food there was no evidence of increase of neoplastic lesions, early onset of neoplasms, increases of rare neoplasms, and other potential carcinogenic findings in males or females.

Table 86 Adrenal weight findings in mice administered cyflumetofen for 18-mo (group means) (Yoshida, 2013)

Dose (mg/kg food)	Weight (mg)	% of Cntl	Relative Wt	% of Cntl
MALE				
Control	7.6	100	0.017	100
10000	7.5	99	0.016	94
FEMALE				
Control	11.8	100	0.026	100
10000	15.8**	139	0.037**	142

*, $p \leq 0.05$; **, $p \leq 0.01$ (Fisher's exact probability test).

Table 87 Incidence of Selected non-neoplastic Adrenal histopathological findings in mice administered cyflumetofen for 18-mo (group means) (Yoshida, 2013)

	MALE		FEMALE	
Dose (ppm)	0	10000	0	10000
	n/N[a]	n/N[a]	n/N[a]	n/N[a]
Adrenal				
Animals killed by design				
Deposition, brown pigment, cortico-medullary junction	3/32	8/35	25/49	31/35**
Hypertrophy, cortical cell, focal	5/32	5/35	0/49	0/35
Vacuolation, cortical cell, diffuse	0/32	18/35**	1/49	28/35**
Hyperplasia, cortical cell, focal	4/32	1/35	1/49	0/35
Hyperplasia, medullary cell	0/32	0/35	0/49	1/35
Hyperplasia, subcapsular cell	15/32	21/35	46/49	28/35
Animals killed *in extremis*, found dead				
Deposition, brown pigment, cortico-medullary junction	3/20	6/17	2/3	13/17
Hypertrophy, cortical cell, focal	2/20	1/17	0/3	0/17
Vacuolation, cortical cell, diffuse	0/20	3/17	1/3	13/17
Hyperplasia, cortical cell, focal	3/20	1/17	0/3	0/17
Hyperplasia, subcapsular cell	7/20	8/17	3/3	12/17
Animals killed by design, killed *in extremis*, found dead				
Deposition, brown pigment, cortico-medullary junction	6/52	14/52*	27/52	44/52**
Hypertrophy, cortical cell, focal	7/52	6/52	0/52	0/52
Vacuolation, cortical cell, diffuse	0/52	21/52**	2/52	41/52**
Hyperplasia, cortical cell, focal	7/52	2/52	1/52	0/52
Hyperplasia, medullary cell	0/52	0/52	0/52	1/52
Hyperplasia, subcapsular cell	22/52	29/52	49/52	40/52*
Spleen				
Animals killed by design				
Haematopoiesis, extramedullary, increased	7/32	3/35	5/49	6/35
Animals killed *in extremis*, found dead				
Haematopoiesis, extramedullary, increased	8/20	9/17	0/3	8/17
Animals killed by design, killed *in extremis*, found dead				
Haematopoiesis, extramedullary, increased	15/52	12/52	5/52	14/52*

* p<0.05; ** p<0.01 Fisher's exact probability test

[a] n = number of of animals with histopathological finding N = Number examined

104-week carcinogenicity study in rats (Takahashi, 2013)

reference	:	Takahashi, 2013	exposure	:	78-weeks, diet
type of study	:	104-week carcinogenicity study	doses	:	: 0, 6000 mg/kg food[1]
year of execution	:	2011-2013	vehicle	:	None
test substance	:	OK-5101; purity 97.82%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 451
species	:	Rat, F344/DuCrlCrlj	acceptability	:	Acceptable
group size	:	50/sex/dose	**NOAEL**	:	<220 mg/kg bw/d (M)
					< 287 mg/kg bw/d (F)

[1] corresponding to 0 and 220 mg/kg bw/d for males and 0 and 287 mg/kg bw/d for females

In a 104 week carcinogenicity study Fisher rats were exposed to cyflumetofen (purity 97.82%) at dietary levels of 0 or 6000 mg/kg food. The dose levels were equal to 0 and 220 mg/kg bw/d for males and 0 and 287 mg/kg bw/d for females. The study was performed according to OECD 451.

120

Toxicologically relevant effects were noted in rats treated with cyflumetofen at 6000 mg/kg food for 104 weeks (see Table 88 to Table 92).

No effect on mortality was observed. Clinical signs in the females were an increased loss of tactile hair as well as an increase in soiled fur in the genital region. Body weights were decreased in weeks 76 to 104 in the males (up to 7% reduction) and consistently throughout treatment for the females (up to 5% reduction) (Table 88). Food consumption was increased at several periods throughout treatment in both the males and females. Haematology findings included decreased lymphocytes, neutrophils, monocytes and eosinophils in the male (Table 89). Gross findings of note included increased testicular masses in the males as well as an increased incidence of atrophy of the epididymis.

Multiple organ weights were affected. Organs with minor though statistically significant changes include increased weights of the brain (male), lung (male), liver (male and female) and kidneys (male and female). Adrenal and testes weights were dramatically increased in both males and females.

Table 88 Mean body weight of rats administered cyflumetofen for 2 years (Takahashi, 2013)

	MALE		FEMALE	
Dose level [ppm]	0	6000	0	6000
Body weight [g]				
Day 0	107	107	87	87
Week 13	312	312	176	170**
Week 52	414	411	219	213*
Week 104	427	398**	281	272
Week 104 Body Weight (% of Control)	-	93.2	-	96.8
Overall body weight gain [g]				
Day 0 to Week 104 bw gain	320	291	194	185
0 – 104 Gain (% of control)	-	90.1	-	95.4

* p<0.05; ** p<0.01

Table 89 Selected haematology findings in rats administered cyflumetofen for 2 years (group means) (Takahashi, 2013)

Dose (ppm)	Lymphocyte (L) $10^3/\mu L$	(L) % of Control	Eosinophil (Eo) $10^3/\mu L$	(Eo) % of Control	Monocyte (Mono)	Mono % of Control	Neutrophil	Neutrophil (% of Cntl)
MALE								
Control	2.46	-	0.07	-	0.26	-	2.25	-
6000	2.01	82*	0.06*	80*	0.21	81*	1.80	86**
FEMALE								
Control	1.49	-	0.04	-	0.20	-	1.04	-
6000	2.44	164	0.04	104	0.12	60	1.08	100

Student t test or Aspin-Welch test: **p<0.01; * p<0.05

Table 90 Selected organ weight findings in rats administered cyflumetofen for 2 years (group means) (Takahashi, 2013)

Dose (ppm)	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (g)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl
	Parameter											

Dose (ppm)	Weight (mg)	% of Cntl	Relative Wt	% of Cntl	Weight (g)	% of Cntl	Relative Wt	% of Cntl	Weight (mg)	% of Cntl	Relative Wt	% of Cntl
	Testes / Ovary				Liver				Adrenal[a]			
MALE												
Control	2735	-	0.68	-	9.73	-	2.39	-	64.5	-	0.016	-
6000	5487	201**	1.44	212**	9.88	102	2.61	109**	85.0	132**	0.023	144**
FEMALE												
Control	69.5	-	0.026	-	6.05	-	2.30	-	68.8	-	0.026	-
6000	89.9	129**	0.035	135**	6.44	106*	2.49	108**	115.8	168**	0.045	173**

$* p \leq 0.05; ** p \leq 0.01$
[a] adrenals in the control and the treatment group that contain spontaneous adenomas or pheochromocytomas (which greatly increase the adrenal weights) are not included in the mean calculations. See the study report for detailed analysis.

Non-neoplastic histopathological lesions included a statistically significant increase in the atrophy of the male pancreas acinar cell (focal). The testes showed decreased hyperplasia of the interstitial cell and a decreased atrophy of the seminiferous tubule. This is considered to be due to the increased incidence of leydig cell tumours seen in the high dose compared to the concurrent control. The adrenal findings included statistically significant increased hypertrophy (cortical cell, diffuse) as well as an increased vacuolation (cortical cell focal and diffuse (female only)). Non-neoplastic (histo) pathological findings are summarized in Table 91 and Table 92.

Table 91 Selected Gross findings (only those with statistically significant incidence) in Animals killed by design (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Animals killed by design				
Systemic/external appearance: [N] Soiled fur, external genital	[41] 1	[43] 1	[39] 2	[38] 11**
Testis: [N] Atrophy Mass(es) Softening	[41] 7 31 16	[43] 1* 41** 2**		
Epididymis: [N] Atrophy	[41] 34	[43] 42*		
Animals killed in extremis or found dead				
Systemic/external appearance: [N] Soiled fur, external genital	[9] 4	[7] 1	[11] 4	12] 6
Testis: [N] Atrophy Mass(es) Softening	[9] 5 3 3	[7] 0* 7* 0		
Epididymis: [N] Atrophy	[9] 6	[7] 6		
All animals examined				

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Systemic/external appearance: [N] Soiled fur, external genital	[50] 5	[50] 2	[50] 6	[50] 17**
Testis: [N] Atrophy Mass(es) Softening	[50] 12 34 19	[50] 1** 48** ⇓2**		
Epididymis: [N] Atrophy	[50] 40	[50] 48*		

[N=]: Number of animals examined.

Figures show the number of animals with findings.

* p ≤0.05; ** p ≤0.01 (Fisher's exact probability test)

Table 92 Incidence of selected histopathological findings in rat administered cyflumetofen for 2-years: All animals examined (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Testis:				
Atrophy, seminiferous tubule	27/50	5/50**		
Hyperplasia, interstitial cell	19/50	3/50**		
Pancreas: Atrophy, acinar cell, focal	15/50	27/50*	5/50	2/50
Thyroid:				
Hyperplasia, C-Cell	17/50	18/50	16/49	14/50
Adrenal: Hypertrophy, cortical cell, diffuse	7/50	44/50**	5/50	39/50**
Vacuolation, cortical cell, diffuse	12/50	7/50	3/50	23/50**
Vacuolation, cortical cell, focal	6/50	15/50*	12/50	24/50**

Fisher's exact probability test: **p<0.01; * p<0.05.

Neoplastic findings included an increased incidence of interstitial cell benign adenoma of the testis (Leydig cell tumour) with statistical significance. This incidence was also outside the range of recent (5-year) historical controls (Table 97). This Leydig cell tumour incidence was determined to be related to treatment.

An increased incidence of C-cell carcinoma of the thyroid gland occurred in the males but not in females. The increased incidence of C-cell carcinoma in males (31% vs 18% in control animals) was not significant, but clearly exceeded the recent (5y) historical control range (6-18%). Further, the incidence of combined C-cell adenoma/carcinoma upon treatment with cyflumetofen (57%) was statistically significantly increased in comparison with concurrent controls (38%), and also slightly exceeding the upper levels of recent (5y) historical controls (26-50%). The increased incidence of carcinoma and combined adenoma/carcinoma in males was only observed when all animals were included (i.e. animals subjected for scheduled kill at week 104 and animals killed *in extremis*). Only when the animals killed in extremis are added (n=2 in control and n=3 in treated animals) statistical significance was attained (p-value=0.04378). The statistically significant increase in males of the combined C-cell adenoma/carcinoma is considered a substance-related effect as this combination is also outside the historical control values.

Data on Leydig cell tumours and C-cell carcinoma after scheduled kill, animals killed in extremis, and all animals are given in Table 93, Table 94 and Table 95.

NOAELs exist for the Leydig cell tumours (NOAEL = 1500 ppm) as well as for all other treatment related effects observed in this study and are found in a prior cancer study conducted at lower doses (Yoshida, 2004f).

For a further evaluation of these neoplastic lesions, reference is made to paragraph 4.10.4. For the testis and thyroid, the historical control data of the performing laboratory IET in the period 1993 – 2013 are given in Table 97, Table 99 Table 100.

Table 93 Animals subjected to scheduled kill after 104 weeks of treatment (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Testis: Tumour, interstitial cell	[41] 33	[43] ↑41		
Thyroid gland: Adenoma, C-cell Carcinoma, C-cell Adenoma/carcinoma, C-cell	[41] 11 7 17	[43] 14 13 25	[39] 7 2 9	[38] 4 2 5

[N=]: Number of animals examined.

Figures show the number of animals with findings.

↑↓, p ≤0.05; ⇑⇓, p ≤0.01 (Fisher's exact probability test).

Table 94 Animals killed *in extremis* and found dead (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Testis: Tumour, interstitial cell	[9] 5	[7] 7		
Thyroid gland: Adenoma, C-cell Carcinoma, C-cell Combined adenoma/carcinoma, C-cell	[9] 0 2 2	[6] 1 2 3	[10] 2 0 2	[12] 1 2 3

[N=]: Number of animals examined.

Figures show the number of animals with findings.

↑↓, p ≤0.05; ⇑⇓, p ≤0.01 (Fisher's exact probability test).

Table 95 All animals examined (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
Testis: Tumour, interstitial cell	[50] 38	[50] ⇑48		
Thyroid gland: Adenoma, C-cell Carcinoma, C-cell Adenoma/carcinoma, C-cell	[50] 11 9 19	[49] 15 15 ↑28	[49] 9 2 11	[50] 5 4 8

[N=]: Number of animals examined.
Figures show the number of animals with findings.
↑↓, p ≤0.05; ⇑⇓, p ≤0.01 (Fisher's exact probability test).
C-cell adenoma/carcinoma represents the combined incidence of both tumours. It is counted as one when both tumours are found in the same animal (Animal Nos. 28, 80, and 95).

Table 96 Overall incidence of microscopic neoplastic lesions in male and female rats administered cyflumetofen for 2-years. All animals examined (Takahashi, 2013)

Organs and lesions	Sex and dose level (ppm)			
	MALE		FEMALE	
	0	6000	0	6000
No. of neoplasms	122	130	71	73
No. of benign neoplasms	97	100	57	54
No. of malignant neoplasms	25	30	14	19
No. of animals with neoplasm(s)	49	50	39	40
No. of animals with benign neoplasm(s)	45	50	35	34
No. of animals with malignant neoplasm(s)	19	23	13	17

Table 97 F344/DuCrj rat: Interstitial cell tumour incidence in the testis in historical control data at IET labs (1993-2012)

Study	Year											
	1993	1994	1995	1997	1998	1999	2003	2005	2008	2009	2011	2012
Study 1	43/50 86%	42/50 84%	43/50 86%	49/50 98%	39/50 78%	35/50 70%	43/50 86%	42/50 84%	36/50 72%	31/50 62%	28/50 56%	36/50 72%
Study 2	42/50 84%	48/50 96%	42/50 84%	48/50 96%	40/50 80%		43/50 86%	41/50 82%				
Study 3	44/50 88%	46/50 92%			39/50 78%							
Study 4		50/50 100%			34/50 68%							

Year: Year at termination of treatment.

Table 98 Cyflumetofen 2-year rat cancer studies: Incidence of Thyroid lesions in all male animals (low dose: Yoshida 2004f, high dose: Takahashi 2013)

Thyroid	0 ppm Low dose Study	0 ppm High Dose Study	150 ppm Low dose Study	500 ppm Low dose Study	1500 ppm Low dose Study	6000 ppm High dose Study
Hyperplasia, C-cell	24/50	17/50	2/23	4/15	17/50	18/49 [#]
Adenoma, C-cell [Be]	9/50	11/50	8/23	4/15	11/50	15/49
Carcinoma, C-cell [Ma]	6/50	9/50	7/23	2/15	4/50	15/49
Combined Incidence of C-cell Adenomas and Carcinomas	14/50 [1]	19/50 [1]	15/23	6/15	14/50 [1]	28/49*[1,2]

[1] Adenoma/carcinoma, C-cell represents the combined incidence of both tumours. It is counted as one when both tumours are found in the same animal.
[2] Animals 80 and 95 had both C-Cell adenoma and C-Cell Carcinoma recorded. The combined incidence calculation counted these incidences once.
* $P \leq 0.05$; exact p-value for this dose group is 0.04378 (one-sided Fischer's exact test)
# it is noticed that in table 79, the incidence of C-cell hyperplasia is presented as 18/50

Table 99 F344/DuCrj rat: C-cell thyroid tumours in historical control data (1997~2013) males

Organs and lesions	Study ID	A	B	C	D	E	F	G	H
	Year	1997	1997	1998	1998	1998	1998	1999	2003
	N	50	50	50	50	50	50	50	50
Thyroid: Adenoma, C-cell (%) Carcinoma, C-cell (%)		8 (16) 0 (0)	8 (16) 0 (0)	20 (40) 2 (4)	13 (26) 4 (8)	8 (16) 0 (0)	14 (28) 4 (8)	12 (24) 2 (4)	9 (18) 5 (10)

Organs and lesions	Study ID	I	J	K	L	M	N	O	P	Q
	Year	2003	2005	2005	2008	2009	2009	2011	2012	2013
	N	50	50	50	50	50	50	50	50	50
Thyroid: Adenoma, C-cell (%) Carcinoma, C-cell (%)		9 (18) 6 (12)	16 (32) 3 (6)	20 (40) 2 (4)	21 (42) 7 (14)	22 (44) 3 (6)	10 (20) 5 (10)	14 (28) 9 (18)	18 (36) 7 (14)	11 (22) 9 (18)

Year: Year at termination of treatment.
N: Number of animals examined.

Table 100 C-cell thyroid tumours (animals with adenoma and/or carcinoma) in historical control data of IET (2005~2013), Males

Organs and lesions	Study ID	A	B	C	D	E	F	G	H
	Year	2005	2005	2008	2009	2009	2011	2012	2013
	N	50	50	50	50	50	50	50	50
(Animals subjected to scheduled kill after 104 weeks of treatment) Adenoma, C-cell		13	16	20	21	7	13	14	11
Carcinoma, C-cell		3	2	5	3	5	8	3	7
Adenoma/carcinoma, C-cell		16	18	22	24	10	19	15	17

(Animals killed in extremis and found dead)								
Adenoma, C-cell	3	4	1	1	3	1	4	0
Carcinoma, C-cell	0	0	2	0	0	1	4	2
Adenoma/carcinoma, C-cell	3	4	3	1	3	1	6	2
(All animals examined)								
Adenoma, C-cell	16	20	21	22	10	14	18	11
Carcinoma, C-cell	3	2	7	3	5	9	7	9
Adenoma/carcinoma, C-cell	19	22	25	25	13	20	21	19

Year: Year at termination of treatment.
N: Number of animals examined.
Adenoma/carcinoma, C-cell represents the combined incidence of both tumours. It is counted as one when both tumours are found in the same animal.

Table 101 Percent Incidence of microscopic neoplastic lesions rats administered Cyflumetofen for 2-years. (Takahashi, 2013)

	Dose (ppm)					
	0	0[a]	150	500	1500	6000[a]
All Animals	86%	76%	86%	90%	92%	96%**
Terminal Kill	98%	80%	95%	95%	98%	95%**
Killed in Extremis / found dead	25%	56%	50%	57%	67%	100%

[a] High-dose study conducted at dose levels of 0 and 6000 ppm

An overall evaluation of the thyroid and testes tumour findings is given in paragraph 4.10.4 and 4.10.5.

4.10.1.2   Carcinogenicity: inhalation

No data

4.10.1.3   Carcinogenicity: dermal

No data

4.10.2   Human information

No data

4.10.3   Other relevant information

Mechanistic studies are summarised in section 4.12 Specific investigations.

4.10.4   Summary and discussion of carcinogenicity

Long term toxicity and carcinogenicity was studied in rat and mouse. In rats orally exposed to cyflumetofen during 1 year increased incidence of hyperplasia of interstitial cells of the testis was observed at a dose level of 250 mg/kg bw/day (6000 ppm) (Yoshida 2012, see section 4.7), whereas no neoplastic lesions were observed at earlier sacrifice times after treatment with the high dose or at any time after treatment with up to and including 56.8 and 69.2 mg/kg bw/day for males and females, respectively (Yoshida 2014e, see section 4.7). Oral exposure of rats during 2 years resulted

in an increased incidence in Leydig cell tumours at a high dose level of 220 mg/kg bw/day compared with the concurrent control and recent 5-y historical controls. In addition, high dose males also showed a tendency to increase in the incidence of C-cell carcinoma in the thyroid (Takahashi 2013). Important to note is that there was no increase in mortality in the high dose group. There were no significant increases in the incidence of any neoplastic lesions in females. At lower doses up to and including 49.5 mg/kg bw/day no increased incidence in Leydig cell tumours and no increased incidence of C-cell carcinoma in the thyroid were observed (Yoshida 2004f).

In mice exposure to cyflumetofen during 18 months did not induce neoplastic lesions up to and including 1143 or 1132 mg/kg bw/day in males and females, respectively, the highest dose tested (Yoshida 2004g, Yoshida 2013).

Leydig cell tumours

Cyflumetofen caused an increased incidence of Leydig cell tumours (LCT, also indicated as Interstitial cell tumours of the testes) at 6000 ppm (220 mg/kg bw/day) in a carcinogenicity study with the Fischer F344/DuCrlCrlj rat (Takahashi 2013), but these LCTs are not related to increased mortality. The LCT incidence was higher than concurrent and recent (5yr) historical control. There was no statistically significant increase in LCT in Fischer F344 rats at the lower doses tested (150, 500 and 1500 ppm) (Yoshida 2004f).

Potential mechanism and human relevance of rat Leydig cell tumours:

Based on the mutagenicity tests, the mechanism for the carcinogenic effect of cyflumetofen is probably non-genotoxic. It is known that some mechanisms for carcinogenicity as observed in test animals are considered not relevant for humans. Leydig cell tumours induced by dopamine antagonists or gonadotropin-releasing hormones (GnRH) are considered not relevant for humans (according to section 3.6.2.2.6-k of the CLP Guidance). These specific mechanisms are not demonstrated for cyflumetofen-induced Leydig cell tumours. However, it is known that some tumour types occur with a high spontaneous tumour incidence or are not relevant for humans. Leydig cell tumours are observed with a high spontaneous tumour incidence in male F344 rats (according to section 3.6.2.2.6-a of the CLP Guidance). Leydig cell tumours are extremely common in the F344 rat, with a reported background[1] incidence of 75-100% in 2-year cancer studies. Humans are orders of magnitude less sensitive with a LCT background incidence of 0.00004% to 0.01%. Most documented instances of LCT in humans occur as a result of a disease state.

The reasons for the different background incidence rate for LCT among species stem from both quantitative and qualitative differences in the Leydig cell response to hormonal stimuli. The rat leydig cell is extremely sensitive to slight changes in circulating luteinizing hormone (LH) levels. Among rat strains, the F344 rat is particularly susceptible to this process and is not considered a relevant model for studying LCT. Because of the differences present between the human and the rat Leydig cells, non-genotoxic compounds that cause LCT in rats have low relevance to humans. This has been confirmed already in 2004 when the Specialized Experts Meeting of the European Chemicals Bureau agreed that "data on LCTs generated in Fisher rats or other rat strains having

---

[1] Boorman GA, Chapin RE, and Mitsumori K (1990).Testis and epididymis. In: *Pathology of the Fischer Rat—Reference and Atlas*, GA Boorman, SL Eustis, MR Elwell, CA Montgomery, and WF MacKenzie (eds). Academic Press, New York, San Diego, pp. 405–418.

comparably high spontaneous LCT rate are considered normally not informative." (ECBI/08/04 Add 4, 2004).

The data with cyflumetofen supports this species and strain-specific effect on the Leydig Cells. Other than at a high dose with the F344 rat, there was no treatment related effects observed on the Leydig cell in another tested rat strain (Wistar) and other species (dog (though very low number of animals evaluated) and mouse), no matter the dose level or the duration of dosing.

The Leydig cell tumours are therefore not taken into account for classification of cyflumetofen with regard to carcinogenicity.

C-cell carcinoma – thyroid

In male rats treated with a high dose of 6000 ppm (220 mg/kg bw/day for males) for a time span of two years an increased incidence of C-cell carcinomas of the thyroid was observed that was not statistically significant but above the concurrent and recent (5y) historical control data. There was no increase in either C-cell hyperplasia or C-cell adenoma at this dose. Statistical significance was obtained only for the combined incidence of C-cell adenomas/carcinomas. The increased incidence of carcinoma and combined adenoma/carcinoma in males was only observed when all animals were included (i.e. animals subjected for scheduled kill at week 104 and animals killed *in extremis*) (Takahashi 2013). The statistically significant increase in males of the combined C-cell adenoma/carcinoma is considered a substance-related effect as this combination is also outside the historical control values.

Potential mechanism and human relevance of rat thyroid C-cell tumours:

Based on the mutagenicity tests, the mechanism for the carcinogenic effect of cyflumetofen is probably non-genotoxic. It is known that some mechanisms for carcinogenicity as observed in test animals are considered not relevant for humans. Certain thyroid tumours in rodents mediated by UDP glucuronyltransferase (UGT) induction are considered not relevant for humans (according to section 3.6.2.3.2 of the CLP Guidance). For cyflumetofen, mechanistic studies investigating the cyflumetofen induced thyroid tumours are not available and this specific mechanism (i.e. UGT induction) is therefore not demonstrated.

The cyflumetofen-induced C-cell carcinoma, as observed in the rat at high dose, was only observed in male animals. This sex-difference could however not be explained by the available data on ADME/TK (see section 4.1 of current CLH-report), as these do not point towards potential higher internal exposure in males. The cyflumetofen-induced C-cell carcinoma could not be demonstrated in mice (even at dose levels as high as those inducing thyroid tumours in rat) which might point towards a species-specificity for the rat. However, no data are available which might further support this.

It is noticed that the increased incidence in the high dose males could not be predicted from any of the repeated-dose experiments performed before. In none of the rat studies from 28-day up to 2-year treatment (i.e. using shorter exposure durations or lower dose levels) were any lesions or histopathological effects of the thyroid recorded. The same is true for all other species cyflumetofen was tested in. Some changes in thyroid weight were however observed in rat, mouse and dog (Takahashi 2004, Yoshida 2004b+d, Nagashima 2003b). These organ weight changes included ↑ thyroid weight (abs/rel) in F_0 females in a rat 2-generation study (Takahashi 2004), a dose-related decrease in thyroid weight (abs/rel) in a 28-d mouse study (Yoshida 2004b), dose-related increase

in thyroid weight (abs) in a 13-wk mouse study (males) (Yoshuda 2004d), and an increase in thyroid weight (abs/rel) in a 13-wk dog study (females, only mid dose) (Nagashima 2003b).

It is noticed that the control male animals have a relatively high background levels of C-cell hyperplasia (34%), adenoma (22%) and carcinoma (18%). This may suggests that this could be enhancement of the spontaneous occurrence levels. The relevance of this effect to humans can be doubted. As no information is available regarding the mechanism of the spontaneous C-cell tumours and its relevance to humans, these tumour types should be considered for classification.

Taking all the data together, a potential irrelevance for humans is not demonstrated for the cyflumetofen-induced thyroid tumours in rat.

4.10.5   Comparison with criteria

The CLP criteria for classification as a category 1 Carcinogen are as follows:

"A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:

Category 1A, known to have carcinogenic potential for humans, classification is largely based on human evidence, or

Category 1B, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

The classification in Category 1A and 1B is based on strength of evidence together with additional considerations (see section 3.6.2.2). Such evidence may be derived from:

- human studies that establish a causal relationship between human exposure to a substance and the development of cancer (known human carcinogen); or

- animal experiments for which there is sufficient (1) evidence to demonstrate animal carcinogenicity (presumed human carcinogen).

In addition, on a case-by-case basis, scientific judgement may warrant a decision of presumed human carcinogenicity derived from studies showing limited evidence of carcinogenicity in humans together with limited evidence of carcinogenicity in experimental animals."

The CLP criteria for classification as a category 2 Carcinogen are as follows:

"Substances are classified as a category 2 Carcinogen when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."

Classification in category 1A is not considered for cyflumetofen as there are no human data regarding carcinogenicity.

Classification in category 1B is not considered for cyflumetofen. The available data cannot be considered as sufficient evidence which is defined as "an increased incidence of malignant

neoplasms or of an appropriate combination of benign and malignant neoplasms in two or more species" (CLP Regulation, section 3.6.2.2.3.b) as an increase in only one study in one species was observed. These criteria are not met.

Classification in category 2 is required when there is limited evidence which is defined as "the data suggest a carcinogenic effect but are limited for making a definitive evaluation because, e.g. (a) the evidence of carcinogenicity is restricted to a single experiment; (b) there are unresolved questions regarding the adequacy of the design, conduct or interpretation of the studies; (c) the agent increases the incidence only of benign neoplasms or lesions of uncertain neoplastic potential; or (d) the evidence of carcinogenicity is restricted to studies that demonstrate only promoting activity in a narrow range of tissues or organs" (CLP Regulation, section 3.6.2.2.3.b).

Based on the available mutagenicity tests, the mechanism for the carcinogenic effect of cyflumetofen is probably non-genotoxic.

An increased incidence in Leydig Cell tumours was observed in cyflumetofen-treated Fischer F344 rats. It is known that Leydig cell tumours are observed with a high spontaneous tumour incidence in male Fischer F344 rats (according to section 3.6.2.2.6-a of the CLP Guidance). Further, the Specialized Experts Meeting of the European Chemicals Bureau agreed in 2004 that "data on LCTs generated in Fisher rats or other rat strains having comparably high spontaneous LCT rate are considered normally not informative." (ECBI/08/04 Add 4, 2004). Therefore, the increase in LCTs is not taken into account for classification for carcinogenicity.

An increased incidence (not statistically significant) in thyroid C-cell carcinoma was observed in cyflumetofen-treated Fischer F344 rats. The increased incidence was above the incidences of recent (5y) historical controls. Further, a statistically significant increased incidence in thyroid C-cell carcinoma/adenoma was observed in cyflumetofen-treated Fischer F344 rats, which was above the incidences of historical controls. This effect is considered treatment-related and suggests a carcinogenic effect of cyflumetofen. The increase in thyroid C-cell carcinoma/adenoma could be due to stimulation of the high spontaneous rate of these tumours in this strain of rats. However, this is not shown. As it cannot be excluded that the observed thyroid tumours are relevant for humans, these should be taken into account for classification of cyflumetofen for carcinogenicity in humans.

Given that the evidence of carcinogenicity is restricted to a single experiment and the potential relevance for humans can be questioned, the irrelevance is not demonstrated for the cyflumetofen-induced thyroid tumours in rat, it can therefore be concluded there is limited evidence for carcinogenic effects of cyflumetofen. Classification for carcinogenicity as <u>Carc. 2 (H351: Suspected of causing cancer)</u> is therefore proposed.

4.10.6 Conclusions on classification and labelling

Classification of cyflumetofen for carcinogenicity as <u>Carc. 2 (H351: Suspected of causing cancer)</u> is required.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

Long term toxicity and carcinogenicity was studied in rat and mouse (table 69, CLH report). The DS summarised two oral GLP and OECD TG 451 compliant carcinogenicity studies along with two supplementary (considered such because only one high dose level was tested) carcinogenicity studies;

- one 18 month study in CD-1 mice (Yoshida, 2004) supplemented with a GLP OECD TG 451 compliant single high dose level study (Yoshida, 2013)

- one 24 month study in Fischer F344 rats (Yoshida, 2004) supplemented with a GLP OECD TG 451 compliant single high dose level study (Takahashi, 2013).

The latest rat and mouse carcinogenicity studies investigated a single high dose level only. They were designed to complement the existing older carcinogenicity studies (which tested cyflumetofen at lower doses) and satisfy US EPA requirements. All cancer studies (old and new) were conducted by The Institute of Environmental Toxicology (IET, Japan), which also supplied the historical control data (HCD).

Non-neoplastic changes in mice

Cyflumetofen (OK-5101, 97.67%) was administered to SPF ICR (Crj:CD1) mice (52/sex/dose) in the diet with mean dose levels up to 537 mg/kg bw/day in the first study and up to 1143 mg/kg bw/day in the supplementary study:

Table: Achieved doses (mg/kg bw/day)

Study:	Yoshida, 2004				Yoshida, 2013	
Males:	0	15.5	54.3	156	537	0	1143
Females:	0	14.3	48.1	144	483	0	1132

Mortality, clinical signs, body weight, food consumption and haematology were not affected by treatment. A substance related increase in the incidence of diffuse vacuolation of adrenal cortical cells was noted in mice treated at ≥ 483 mg/kg bw/day (tables 84, 87, CLH report). There was no evidence of hypertrophy (in contrast with rats). This was statistically significant and outside the historical control range. At 1132 mg/kg bw/day, females showed significant increases in the incidences of pale-coloured skin and pale-coloured eye/eyelid. In organ weight, females showed statistically significant increases in absolute and relative weights of the adrenals (table 86, CLH report, +39, +42% relative to controls respectively). The incidence of oligospermia was increased relative to controls at the highest dose only (15/52 vs 24/52).

Neoplastic changes in mice

There was no evidence of an increase in neoplastic lesions, early onset of neoplasms, increases of rare neoplasms, or other potential carcinogenic findings following exposure to cyflumetofen at doses up to 1143 and 1132 mg/kg bw/day in males and females, respectively.

Non-neoplastic changes in rats

Cyflumetofen (OK-5101, 97.67%) was administered to SPF Fischer 344 (F344/DuCrj) rats (50/sex/dose) in the diet with mean dose levels up to 61.9 mg/kg bw/day in the first study and up to 287 mg/kg bw/day in the supporting supplementary carcinogenicity study:

Table: Achieved doses (mg/kg bw/day)

Study:	Yoshida, 2004				Takahashi, 2013	
Males:	0	4.92	16.5	49.5	0	220
Females:	0	6.14	20.3	61.9	0	287

No effects on mortality, clinical signs, and body weight or food consumption were noted. Absolute and relative adrenal and ovary weights in females at ≥ 61.9 mg/kg bw/day were increased. A substance related statistically significant increase in the incidence of diffuse/focal vacuolation of cortical cells (with strong evidence of hypertrophy) in the adrenals was noted in female rats treated at the highest dose of 287 mg/kg bw/day (table 92, CLH report). Adrenal and ovary weights were increased in females at the highest dose (table 90, CLH report).

Neoplastic changes in rats

In male F344 rats, cyflumetofen exposure correlated with an increased incidence of Leydig cell tumours (LCT, also indicated as Interstitial cell tumours of the testes); and of C-cell carcinomas of the thyroid above the concurrent and historical control data at the highest dose tested, 220 mg/kg bw/day, without an increase in mortality at that dose. Based on the mutagenicity tests, the DS considered that the mechanism for the carcinogenic effect of cyflumetofen was non-genotoxic.

Although LCT can be induced by a number of mechanisms such as dopamine antagonists or gonadotropin-releasing hormones (GnRH), these are generally considered not relevant for humans (see CLP Guidance). The DS noted that these specific mechanisms and others have not been explored or demonstrated for cyflumetofen-induced LCT. In the CLP Guidance it is stated that these tumours are typically observed with a very high spontaneous tumour incidence in male F344 rats. The DS argued that data from studies with cyflumetofen support a species and strain-specific effect on the Leydig cells which could be explained by both quantitative and qualitative differences in the Leydig cell response to hormonal stimuli. Hence, the DS did not include LCT in the assessment of cyflumetofen for classification for carcinogenicity.

There was also an increased incidence of C-cell carcinomas of the thyroid observed in male rats at the highest dose only (220 mg/kg bw/day). There was no increase in C-cell hyperplasia. There was a non-statistical increase in C-cell adenoma at the high dose relative to controls, and statistical significance was observed only for the combined incidence of C-cell adenomas/carcinomas, and then only when all animals were included (i.e. animals scheduled for termination at week 104 as well as animals killed in extremis). The DS considered this a substance-related effect.

The DS considered that although induction of the tumour by cyflumetofen could be present, the high spontaneous rate of these tumours in this strain of rats could potentially explain the increase in thyroid C-cell carcinoma/adenoma, however this has not been demonstrated. Since it could not be excluded that the observed thyroid tumours are relevant for humans, the DS considered that these should be taken into account for classification of cyflumetofen for carcinogenicity. The DS therefore concluded that there was limited evidence of carcinogenic effects for cyflumetofen and proposed classification as Carc. 2; H351: Suspected of causing cancer.

Comments received during public consultation

Two MSCA provided comments on this hazard class. Although both MSCA agreed with the DS proposal to classify cyflumetofen as Carc. 2, one MSCA was of the view that the LCT should not be disregarded for classification for carcinogenicity.

Two comments were also received from industry. Both commenters agreed with the conclusion of the DS that the LCT were not relevant to humans, but disagreed that the thyroid tumours could be used for classification (arguing that these were sporadic in nature) and therefore disagreed with the proposal of the DS to classify cyflumetofen for carcinogenicity. To support their position, they provided detailed position papers outlining recent additional investigations that were not available at the time of preparation of the CLH report.

Although in their response the DS agreed that the UGT induction based mode of action can be relevant for follicular cell adenomas/carcinomas, they noted that this was not the case for C-cell carcinomas. They also noted that from the available data it was not likely that the carcinogenicity occurred via a genotoxic mechanism, though they also remarked that such a mechanism can never be completely excluded.

Several documents were submitted by industry during the public consultation. These were mainly position papers presenting their opinion on the results from the long-term rat and mouse studies or concentrating on C-cell proliferation and interpretation of C-cell and LCT. RAC commented on these in more detail in the Background document and reanalysed some of the associated statistics.

Additional key elements

Several documents were submitted by industry during the public commenting period. These were mainly position papers presenting their opinion on the results from the long-term rat and mouse studies or concentrating on C-cell proliferation and interpretation of C-cell and LCT.

Submitted documents

1. *Statement on the impurity profile of Batch 09/0510-3 (009025) used in the MTD studies in rats and mice, Pluijmen, 2014.* A confidential report evaluated the batches used in 2012-2013 against the original specification of cyflumetofen in Vol. 4 of the DAR as agreed during the EU review of the substance (*Pluijmen, 2014*). The report concluded that the levels of impurities in the newer technical material were representative of those in the older batches on which the DAR specification was based.

2. *Retrospective analysis of cell proliferation in the thyroid gland for the study BAS 9210 I (Cyflumetofen): Repeated Dose 1-Year Oral Toxicity Study in Rats. Marxfeld & van Ravenzwaay, 2017.* The potential cell proliferation in C-cells of thyroid glands from male Fischer rats [F344/DuCrlCrlj] was investigated at the 14 and 53 week time points from animals dosed with 0 and 250 mg/kg bw/day cyflumetofen in the dietary 12 month study (*Yoshida, 2012*). Paraffin sections of thyroid glands were stained with antibodies to calcitonin to identify C-cells of the thyroid gland and also with Ki-67 to detect proliferating cells. There was no increased cell proliferation or calcitonin

production in animals treated with cyflumetofen for up to 53 weeks.

3. *Biological Relevance of C-cell tumors in a High Dose 2-yr Rat Cancer Study with Cyflumetofen*. Industry position paper, BASF Doc ID 2017/1053919. This position paper incorporated two consultant reports and outlines many points, concluding that the increased C-cell carcinoma incidence is considered sporadic in nature and does not warrant any classification according to the criteria of CLP regulation.

4. Position Report on the Classification Proposal for cyflumetofen, Charles River final report, 2017. This report outlined the arguments against the proposed classification, and summarised all available relevant information reflecting OAT's (OAT Agrio Co. Ltd.) position regarding the classification proposal for carcinogenicity. Additional information was included, based on the recent report on the staining of thyroid tissue with the proliferative marker Ki67.

RAC notes these specific effects for consideration of classification:

- Thyroid C-cell adenomas ↑ in males at the highest dose:

  [Controls: 9/50; 11/50]; 8/23; 4/15; 11/50; **15/49** (all data from 2004 and 2013 reports)

- Apparent progression to malignancy: thyroid C-cell carcinomas ↑ in males at the highest dose:

  [Controls: 6/50; 9/50]; 7/23; 2/15; 4/50; **15/49**

- There could be a potential under reporting of the incidences of adenomas/carcinomas at the low dose (4.9 mg/kg bw/day) and next dose (16.5 mg/kg bw/day). The reason being that it is not clear if all of the dose group animals were evaluated for histopathology at the time of the original report (2004). The legend implies that only animals with macroscopic thyroid lesions were examined.

A Note on Statistics (using the rat thyroid C-cell data)

RAC recalculated Fisher's Exact test when investigating dose vs C-cell tumour findings. The following parameters were described:

- The nominal variables are dose and C-cell tumours).

- The biological null hypothesis is that the dose does not affect the likelihood of getting cancer.

- The statistical null hypothesis is that the proportions of rats with C-cell tumours are the same for each dose tested.

- Total maximum sample size is 249, and generally if the total sample size is < 1000 we use the Fisher's Exact test rather than the chi-square test.

- It does not matter which variable is used for row or column.

- The chosen level of significance is $p = 0.05$.

- Method used: sum of small p-values (generally preferred by statisticians).

- Fisher's Exact probability test was carried out as a two-tailed analysis.

The DS described the increased incidence of C-cell carcinoma in males (31% vs 18% in control animals) as not being statistically significant. This was in line with the results from the original study report (Takahashi, 2013) that only assessed the tumour incidences at 0 and 220 mg/kg bw/day. A statistical evaluation of the C-cell tumours from both the Yoshida (2004) and Takahashi (2013) combined data was not originally performed.

RAC re-evaluated this data. While it is recognised that grouping sample data from two studies separated by several years is not ideal, it is nevertheless considered appropriate to do so in this case so that all rat C-cell tumour data can be evaluated in a meaningful way. The HCD from the laboratory that performed the carcinogenicity studies using the F344 rats is quite stable and normally distributed over the years 2003 – 2013. Therefore, it is quite reasonable to combine the data from the two carcinogenicity studies to enable a valid comparison amongst all the doses tested.

Incidence of male F344 rat Thyroid C-cell carcinomas 2003 – 2013, IET lab historical controls

Year	2003	2003	2005	2005	2008	2009	2009	2011	2012	2013
Incidence: *Carcinoma, C-cell*	5	6	3	2	7	3	5	9	7	9
n	50	50	50	50	50	50	50	50	50	50

When all the data from the two studies are combined and analysed using Fisher's exact test, the incidence of C-Cell carcinomas is found to be highly statistically significant (p = 0.003).

Statistical evaluation of the incidence of C-cell carcinoma in male rats

(a) Fisher's exact test, 2 × 2 contingency table (control vs high dose group data only)

Disease: C-Cell Carcinoma

Dose	no tumours	tumours	% incidence
0	41	9	18
220	34	15	30.6

Result: p = 0.165 (two-sided) not significant. A 1-sided test is also not significant (p = 0.109), a 1-sided test is appropriate for consideration if we are only concerned with treatment that may result in an increase in carcinomas (i.e. we ignore any decrease in tumours).

(b) Fisher's exact test, 5 × 2 contingency table (*mean of both controls vs all dose group data from 2004 and 2013);

Disease: C-Cell Carcinoma

Dose	no tumours	tumours	% incidence
0*	42	8	16
4.9	43	7	14
16.5	48	2	4
49.5	46	4	8
220	34	15	30.6

Result: p = 0.003 (two-sided) very significant.

Statistical evaluation of the incidence of C-cell adenomas in male rats

A similar analysis for C-Cell adenomas shows no statistical significance at any dose level.

(a) Fisher's exact test, 2 × 2 contingency table (control vs high dose group data only); p = 0.368 (two-sided); p = 0.228 (1-sided) not statistically significant.

(b) Fisher's exact test, 5 × 2 contingency table (all treatment groups from 2004 and 2013); p = 0.062 (two-sided) not statistically significant.

Statistical evaluation of the incidence of C-cell adenomas and carcinomas combined (male rats)

A similar analysis for C-Cell adenomas and carcinomas combined shows a highly significant statistical result whether looking at the Takahashi (2013) data alone in a 2 × 2 contingency table or at all available dose groups in a 5 × 2 contingency table.

(a) Fisher's exact test, 2 × 2 contingency table (control vs high dose group data only); p = 0.0438 (1-sided) significant; p = 0.0711 (2-sided), not significant.

(b) Fisher's exact test, 5 × 2 contingency table (all treatment groups from 2004 and 2013); p = 7×10^{-5} (two-sided), highly statistically significant.

Assessment and comparison with the classification criteria

Technical equivalence of batches used in the long-term studies in rats

IET conducted two carcinogenicity studies in rats with cyflumetofen. One study was conducted at doses up to 62 mg/kg bw/day (Yoshida, 2004), while a second high dose study was conducted at 0 and 220/287 mg/kg bw/day (Takahashi, 2013). The batches of cyflumetofen were very similar, batch 01D1 from the 2004 study was 97.67% pure while batch 09/0510-3 (009025) from the newer technical material was 97.82% pure. A confidential report evaluated the batches used in 2012-2013 against the original specification of cyflumetofen in Vol. 4 of the DAR as agreed during the EU review of the substance (statement on the impurity profile of Batch 09/0510-3 (009025) used in the MTD studies in rats and mice, Pluijmen, 2014). The report concluded that the levels of impurities in the newer technical material were representative of those in the older batches on which the DAR

specification was based.

Relevance of tumours observed in long-term studies in rats

Neoplastic findings included an increased incidence of interstitial cell benign adenoma of the testis (Leydig cell tumour, LCT) with statistical significance at the highest dose (96% vs 76% in control animals). This incidence was also outside the range of recent (5-year) IET HCD (56-76%; mean 68%). It is also noteworthy to point out that <u>all tested doses</u> of cyflumetofen resulted in incidences of LCT outside the range of the IET laboratory HCD without a discernible dose response.

An increased incidence of C-cell carcinoma of the thyroid gland occurred in males but not in females. The increased incidence of C-cell carcinoma in males (31% vs 18% in control animals) was not statistically significant, but clearly exceeded the IET laboratory historical control range (6-18%; mean 14%).

Leydig cell tumours

Table: Incidence of F344 rat interstitial cell tumours, numbers of animals affected

Dose in mg/kg bw/day	0[1]	0[2]	4.9[1]	16.5[1]	49.5[1]	220[2]
All animals examined	43/50	38/50	42/49	43/48	46/50	**48/50****

[1] Yoshida, 2004; [2] Takahashi, 2013; ** $p \leq 0.01$ (Fisher's exact probability test)

Table: Incidence of F344 rat interstitial cell tumours in IET HCD

Year	2008	2009	2011	2012	2013[1]
Incidence (animals affected)	36	31	28	36	38
n	50	50	50	50	50

[1] Takahashi, 2013 control.
Mean: 34 ± 4; range 28 – 38; normally distributed.
Older studies (1993 – 2005) show incidences from 35 – 49 animals affected out of 50.

Table: Incidence of F344 rat interstitial cell hyperplasia in 1 year study, numbers of animals affected, 12 month time point

Dose in mg/kg bw/day	0[1]	0[2]	1.9[1]	5.6[1]	18.8[1]	56.8[1]	250[2]
Animals examined	0/20	6/20	0/20	0/20	0/20	0/20	**19/20****

[1]. Yoshida, 2004; [2]. Yoshida, 2012; ** $p \leq 0.01$ (Fisher's exact probability test)

The Leydig cell tumour incidence was higher than concurrent and HCD. The mutagenicity tests do not support a genotoxic mode of action for cyflumetofen. Hyperplasia is the proliferative precursor lesion of LCT. LCT begin as Leydig cell hyperplasia (LCH), and the distinction between hyperplasia and adenoma is only based upon size (i.e. a mass of interstitial cells with a diameter greater than three seminiferous tubules is considered an adenoma). The only study where an increased incidence of LCH was observed was in the

high dose (250 mg/kg bw/day) 1-year chronic rat study (table Incidence of F344 rat interstitial cell tumours, numbers of animals affected", above). This incidence is increased over the HCD (incidence of 12 month LCH within the last five relevant years at IET is 2/20 – 1/20 – 0/20 – 9/20) and concurrent controls (6/20). When considered along with the early onset of hyperplasia (at the high dose in the 1-year chronic study, Yoshida, 2004), the increased incidence of LCT at the high dose in the 2y cancer study may be considered treatment related, but this is against a significantly high background. Also, it is noted that by the end of the 15 month study period nearly all F344 rats will have either hyperplasia or adenomas (Boorman *et al.,* 1990), so establishing a substance related effect under these conditions must be considered with caution.

Five *in vitro* mechanistic studies were performed to identify a possible mode of action for LCT via perturbation of the oestrogen or androgen system.

1. Aromatase assay: cyflumetofen was not an inhibitor of CYP19 aromatase activity.

2. Oestrogen receptor activation: an *in vitro* human ERα-transcriptional activation system showed cyflumetofen was negative in two out of three assays and only marginally positive (RPCMax: 10.4%) in the remaining assay. Considered negative overall.

3. *In vitro* ER-binding assay: cyflumetofen was considered to be "not interactive" for ER binding.

4. *In vitro* AR binding assay: Marginal displacements of [^{3}H]R1881 (a synthetic androgen, also called methyltrienolone), at concentrations at or near precipitation of cyflumetofen were observed, cyflumetofen was considered to be "not interactive" for AR binding.

5. *In vitro* H295R steroidogenesis assay: cyflumetofen demonstrated an apparent influence on the steroidogenesis pathway, acting as an inducer of estradiol production and an inhibitor of testosterone production in this test system. The biological significance of the small changes in steroid metabolism is considered equivocal, especially in the context of absent significant similar changes or adverse consequences *in vivo*. In addition, if cyflumetofen does act on the adrenal glands to affect hormone sensitive lipase activity then perturbations in other steroidogenic tissues such as testes and ovary are to be expected. The significance of the effects in this assay are therefore considered uncertain.

The mode of action remains unclear. Indeed, it is debateable if it is meaningful in the context of a lesion (LCT) that occurs in the F344 rat where that lesion's background incidence is so high that it is not usually considered relevant to other species, including humans, or even to other rat strains. Generally this is the case for LCT in the Fisher F344 male rat (section 3.6.2.2.6-a of the CLP Guidance). In 2004, the Specialized Experts Meeting of the European Chemicals Bureau agreed that "data on LCTs generated in Fisher rats or other rat strains having comparably high spontaneous LCT rate are considered normally not informative" (ECBI/08/04 Add 4, 2004).

Leydig cell tumours are extremely common in the F344 rat, with a typical background incidence of 75-100% in 2-year cancer studies. Humans are orders of magnitude less sensitive to the development of this tumour type. The F344 rat is particularly susceptible to this process and is not considered a relevant model for studying LCT. Because of the differences present between the human and rat Leydig cells, non-genotoxic compounds that

cause LCT in rats generally have low relevance to humans. RAC therefore considers the LCT incidence for F344 rats dosed with cyflumetofen <u>not to be relevant for classification purposes</u>.

<u>Thyroid C-cell tumours</u>

Table: Incidence of male F344 rat Thyroid C-cell tumours (all animals)

Dose in mg/kg bw/day	0[1]	0[2]	4.9[1]	16.5[1]	49.5[1]	220[2]
Incidence: *Adenoma, C-cell* *Carcinoma, C-cell* *A/C, C-cell*	9 6 14	11 9 19	8 7 15	4 2 6	11 4 14	15 15 **28***
n	50	50	50 (23)	50 (15)	50	49

[1] Yoshida, 2004; [2] Takahashi, 2013

☐ (shaded cells) The incidences reported for doses 4.9 mg/kg bw/day and 16.5 mg/kg bw/day may be underestimates, only 23 and 15 animals examined respectively out of a total of 50 in each dose group, i.e. only animals with macroscopic thyroid lesions were accessed. However, these doses are so low that they may be expected to reflect the normal control incidence. This would appear to be the case.

* $p \leq 0.05$ (Fisher's exact probability test)

Table: Incidence of male F344 rat Thyroid C-cell tumours in IET HCD

Year	2008	2009	2009	2011	2012	2013[1]
Incidence: **Adenoma, C-cell** **Carcinoma, C-cell** **A/C, C-cell**	21 7 25	22 3 25	10 5 13	14 9 20	18 7 21	11 9 19
n	50	50	50	50	50	50

[1] Takahashi, 2013 control.
Adenoma Mean: 16 ± 5; range 10 – 22; normally distributed.
Carcinoma Mean: 7 ± 2; range 3 – 9; normally distributed.
A/C Mean: 21 ± 4; range 13 – 25; normally distributed.

Table: Incidence of male F344 rat Thyroid C-cell hyperplasia

Dose in mg/kg bw/day	0[1]	0[2]	4.9[1]	16.5[1]	49.5[1]	220[2]
Incidence	24	17	2	4	17	18
n	50	50	50 (23)	50 (15)	50	49

[1] Yoshida, 2004; [2] Takahashi, 2013
☐ (shaded cells) The incidences reported for doses 4.9 mg/kg bw/day and 16.5 mg/kg bw/day may be underestimates, only 23 and 15 animals examined respectively out of a

total of 50 in each dose group.

The incidence of thyroid C-cell adenoma in high dose (220 mg/kg bw/day) male rats was greater than the concurrent control levels (15/49 high dose vs 11/50 for the control, Takahashi, 2013), and was not statistically significant (p = 0.125). C-cell adenomas occur frequently and with a large variation in Fisher F344 rats and this may reduce concern with respect to their human relevance. The HCD clearly illustrates that the observed adenomas fall within the normal background (mean 32% or 16/50 animals, range 10 – 22/50) expected from this rat strain observed in the same testing facility as that used to generate the cyflumetofen data. In isolation, the adenoma incidence data do not provide reliable evidence of a treatment related carcinogenicity.

The incidence of thyroid C-cell carcinoma male rats exposed to high dose (220 mg/kg bw/day) was greater than the concurrent control levels (15/49 high dose vs 9/50 for the controls) and also greater than the HCD at the testing institute (mean 14% or 7/50 animals, range 3 – 9/50). A Fisher's exact test performed by RAC at all dose levels was significant (p = 0.003). The key question therefore becomes: is the incidence of C-cell carcinoma at the 220 mg/kg bw/day dose level sufficient to justify classification for carcinogenicity category 2?

The incidence of combined C-cell adenomas and carcinomas is greater than both the concurrent control levels and HCD, with statistical significance.

There is no mechanistic data available to explain the pathogenesis for the development of the C-cell tumours observed in the high dose group. The data indicate that there is a progression towards malignancy; that overall neoplastic potential is associated with the high dose cyflumetofen group only. Limited evidence is thus available in animals to suggest a carcinogenic effect but a causal relationship cannot be stated emphatically. The key information for consideration by RAC is:

1. There is a well conducted single study in one species with one sex affected.

2. Malignant neoplasms (30.6%) occur more frequently than in concurrent controls (18%) and also lie outside of the testing laboratory's historical control data.

3. Benign neoplasms also show an increased incidence in the high dose relative to concurrent controls but this increase is within the HCD of the testing laboratory.

4. The particular tumours of interest (C-cell carcinomas) exhibit a moderate background incidence of 6 – 18% (2008 – 2012).

5. The tumour type is relevant for human hazard assessment (where it is known as Medullary Thyroid Carcinoma, MTC).

RAC considers that the incidence of C-cell carcinoma in the high dose rat carcinogenicity study with cyflumetofen (Takahashi, 2013) to be potentially substance related and this issue is discussed further below.

Treatment related effect or of spontaneous origin: consideration of weight of evidence

Consideration of a substance related effect

There are several important questions to note here, chief amongst them is why is there a lack of other supporting evidence that fits in with a presumed model of carcinogenesis that would normally be expected for a substance mediated effect, i.e. dose dependent

proliferative lesions of C-cells resulting in the progression of focal C-cell hyperplasia to adenoma and thereafter carcinoma with increasing age? It may be because cyflumetofen exerts a subtle but substance-specific mode of action, or it could be due to an unspecific stimulation of these tumours in this strain of rats that results in a high spontaneous rate of tumours, or it could be simply sporadic in nature. In humans, thyroid C-cell tumours give rise to MTC. C-cell hyperplasia may precede and accompany sporadic MTC but it is also frequently absent except in the case of hereditary MTC. This implies there is no guarantee that C-cell hyperplasia will always precede MTC in humans. In the case of subtle effects or less sensitive species, there is no guarantee that C-cell hyperplasia would be a notable feature of tumourigenesis. Methodological factors may also play a part because single random sections through a small organ like the rat thyroid may not record small tumours such as C-cell adenomas (Hardistry, 1985). Serial sectioning of this organ has been shown to improve the detection of a series of rat tumours including thyroid C-cell adenomas (Thompson & Hunt, 1963).

According to CLP Annex I, 3.6.2.2.6, a number of important factors (among others) may be considered which may increase or decrease the level of concern for carcinogenicity and the classification category.

1. Tumour type and background incidence - **Relevant**:
   By default, carcinogenic effects in experimental animals are considered relevant to humans. There is no evidence to indicate that C-cell tumours in rodents are not relevant to humans. Indeed humans exhibit C-cell tumours in the form of medullary thyroid cancer (MTC).

   The background incidence of C-cell carcinoma is moderate in male F344 rats and fairly stable within recent (5-year) IET laboratory historical controls. The effect in the high dose group is both above concurrent controls and greater than the upper bound limit of the HCD. Unlike Leydig cell tumours in this strain of rat, C-cell carcinomas are considered relevant for human hazard assessment. The presence of an increased incidence in tumours supports Category 1B but the moderate background level may reduce the biological significance for this neoplasm in the F344 rat. According to the latest CLH guidance, "… appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification…" In this case expert judgement considers a case for Category 2 or no classification to be plausible.

2. Statistically significant effects in comparison to the concurrent controls? – **Yes**
   Observed for both carcinomas and for the combined incidence of adenomas and carcinomas. Limited evidence for a carcinogenic effect - Category 2.

3. Is there a dose-dependency of the effect? – **No**
   Effects are only seen at the highest dose tested (220 mg/kg bw/day). Limited evidence for a carcinogenic effect - Category 2.

4. Are pre-neoplastic effects observed? – **No**
   No effect on C-cell hyperplasia was seen in any treatment group compared to the controls. No increase in calcitonin or Ki-67 labelling after 14 and 53 weeks of treatment. Low concern for carcinogenicity. No support for classification.

5. Multi-site responses – **No**
   One type of tumour (C-cells) is observed at only one site (thyroid) in one species (rat). This may be sufficient to downgrade a classification from Category 1B to

<u>Category 2</u>.

6. Progression of lesions to malignancy – **Relevant**
   While the increase in C-cell adenomas is not considered significant per se, a treatment related increase in C-cell carcinomas is evident in the Takahashi study (2013) and indicates a progression towards malignancy. This could support <u>Category 1B</u>. In contrast, the lack of precursor proliferative lesions of the C-cell in shorter duration studies, or in studies with lower doses of cyflumetofen supports a spontaneous aetiology. Category 2 or no classification are possible.

7. Reduced tumour latency – **No**
   The data from scheduled interim necropsy and repeated dose studies indicates there is no reduction in tumour latency. <u>No support for classification</u>.

8. Are responses seen in a single sex or both sexes – **One Sex**
   Only males showed an increased incidence of tumours following treatment in a non sex-specific tissue. ADME studies indicate there is no sex specific differences in toxicokinetics. Females received a higher dose (287 mg/kg bw/day) but did not present with a convincing tumour response. Additional data would be required to provide sufficient evidence for animal carcinogenicity (1B), a decreased level of biological significance in this case supports <u>Category 2 or no classification</u>.

9. Are responses seen in a single species or multiple species – **One Species**
   Only male rats from the F344 strain showed an increase in C-cell tumours at the high dose level (220 mg/kg bw/day) over a lifetime study. There was no record of C-cell tumours or C-cell lesions of any kind in the mouse lifetime studies. The mice were dosed up to 5-fold greater (1143/1132 mg/kg bw/day) than the doses used in the rat lifetime studies. Responses observed in a susceptible strain against a moderate spontaneous background supports <u>Category 2 or no classification</u>.

10. Structural similarity to a substance(s) for which there is good evidence of carcinogenicity – **Not Relevant**
    No data.

11. Routes of exposure – **Relevant, Oral**

12. Comparison of ADME between test animals and humans – **No Data**.

13. Possibility of a confounding effect of excessive toxicity at test doses? – **No**
    No effect on mortality was observed in the Takahashi study *(*2013). Body weights were decreased in the males by up to 7%. Body weight gain relative to controls over the entire test period was reduced by about 10%. Adrenal and testes weights were substantially increased. The study was designed to exceed the MTD. Category 2 or <u>no support for classification</u>.

14. Mode of action and its relevance for humans – **Unknown**
    See section 4 below for a more detailed assessment. The mode of action is unknown, but for compounds that induce C-cell tumours there is typically a common pattern of development of pre-neoplastic and neoplastic lesions, i.e. the progression from increased hyperplasia to adenoma and finally carcinoma. In addition, precursor lesions (hyperplasia and adenomas) typically have a higher incidence than the final malignant tumours. These sequences of events were not observed for cyflumetofen. Several mechanisms could be eliminated based on deductive reasoning and a lack of secondary effects. No specific data is available to propose a mode/mechanism of

action. It is unlikely to be vitamin D_3 mediated, no chronic hypercalcaemia, and there is no data to support cyflumetofen acting on or enhancing GLP-1R receptor activity. Category 2 or <u>no support for classification</u>.

15. Supporting evidence from genotoxicity data – **No**.
   Based on the available mutagenicity tests, the mechanism for the carcinogenic effect of cyflumetofen is probably non-genotoxic. <u>No support for classification</u>.

16. Sufficient evidence (Carc. 1B) for carcinogenicity in animals:

   a. Evidence from two or more independent studies in one species carried out at different times or in different laboratories or under different protocols – **No**.

   b. Evidence derived from a clear increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP – **No**.

<u>Consideration of no substance related effect</u>

The case argued against classification and a substance mediated effect has a central tenet; namely, the increased incidence in C-cell carcinomas (Takahashi, 2013). It is most likely not treatment related but spontaneous in nature. This was based on a number of observations inconsistent with the idea of a substance promoted carcinogenic effect on the thyroid C-cells:

- No increased incidence in proliferative precursor lesions (hyperplasia, adenomas),

- The absence of proliferative events was confirmed by retrospective staining of thyroid tissue with the proliferative marker Ki67. No increased proliferation was observed for animals treated 3 months or 1 year with 250 mg/kg bw/day,

- The incidence for C-cell adenoma shows large variations in F344 rats,

- There is no dose response in the incidence of C-cell carcinomas,

- There is no change in tumour latency,

- There is no difference in the unilateral vs bilateral occurrence of C-cell carcinoma between the high dose group and concurrent controls – almost all the C-cell carcinomas that were observed, occurred unilaterally in both groups (12 of 15 treated-male rats vs 7 of 9 males),

- Effects are observed in male rats but not in females in the absence of a relevant sex specific difference in internal dose or toxicokinetics as shown in ADME studies,

- C-cell carcinomas are not seen in mice treated for up to 18 months at 1143/1132 mg/kg bw/day which is approximately 5 times the dose used in the rat lifetime study.

Mode of action (MoA) of C-cell tumour formation

A comprehensive overview of known modes of action for C-cell tumour formation were presented by industry in its public comments and supplied in the form of position papers evaluating the relevance of the C-cell tumours. The MoA is not known for cyflumetofen and was not investigated in any depth. A comparison with inducers known to promote C-cell tumours in rodents (ionizing radiation, chronic hypercalcemia (due to e.g. excess Vitamin D; excess dietary calcium intake or absorption; stimulation of C-cells induced by compounds enhancing GLP-1 receptor activity or acting as direct agonists), indicates that the increase in proliferative lesions of the thyroid follows a standard pattern of progression from hyperplasia

via adenoma to carcinoma.

Disruption of calcium homeostasis

The studies available for cyflumetofen indicated no altered plasma calcium-levels when treated for up to one year. There was no treatment related pathological change in any calcium homeostasis-related organ like bone tissue, kidney or parathyroid. Therefore, a hypercalcemia related background is not considered likely.

GLP-1 receptor agonism of C-cells

Involvement of the GLP-1 receptor cannot be directly ruled out since calcitonin levels were not measured. However, based on a lack of expected secondary effects (altered blood glucose levels or increased incidence of C-cell hyperplasia), therefore this mode of action cannot be considered relevant given the absence of the expected secondary effects.

C-cell hyperplasia as a precursor lesion

It is highly plausible that all possible mechanisms involve C-cell hyperplasia as a precursor lesion to the development of C-cell tumours. None of the studies with cyflumetofen gives any indication of increased incidences of C-cell hyperplasia at any treatment time point. There were no indications of thyroid involvement when cyflumetofen was tested in a comprehensive set of studies in the rat, ranging from 28 days up to 1 year of treatment except for the 2-year study with a high dose level of 220 mg/kg bw/day. A supplemental 1-year toxicity study (Yoshida, 2012), was conducted at 0 and 250/319 mg/kg bw/day in male and female rats, which were killed after 4, 13, and 52 weeks of treatment. There were no proliferative lesions in the thyroid glands of either sex after 4 and 13 weeks of treatment. When the thyroids were examined after 52 weeks of treatment, C-cell hyperplasia was observed in 2 control males, 2 control females and 3 treated-females, and C-cell adenoma was observed in one control male and one treated-female. Of note, no proliferative lesions in the thyroids were found in the treated males.

Gender-specific differences in kinetics or metabolism

An increased incidence in C-cell tumours was only observed in male rats. Based on the available kinetic data from the high dose ADME studies reported in the DAR, female rats are expected to be exposed to higher levels of cyflumetofen or its metabolites than males. Furthermore, little to no difference is seen with respect to tissue distribution or total radioactive label in the thyroid from both sexes. There were no gender-specific differences in kinetics or metabolism to explain why only one sex was effected.

In conclusion, the mode of action for the formation of C-cell tumours in male rats dosed with 220 mg/kg bw/day remains unknown. RAC is of the opinion that thyroid C-cell tumours in the F344 rat cannot be easily disregarded in the context of human hazard assessment.

Comparison with the Criteria

Classification as *Category 1B is not supported,* since there is no firm evidence from animal experiments to demonstrate a strong substance related effect. There was no effect in the mouse, and the only relevant tumours were thyroid C-cell carcinomas in one sex (males) from one study (Takahashi, 2013) with no dose response (high dose effect only) and no substance related increase in pre-neoplastic lesions.

The increase in thyroid C-cell carcinoma could be due to stimulation of the high spontaneous rate of these tumours in this strain of rats. However, this is not shown. In any case, an

increase in the incidence of a spontaneously occurring tumour is still subject to consideration and may be sufficient to downgrade a classification from Category 1B to Category 2, and also be sufficient to consider no classification.

As it cannot be excluded that the observed thyroid tumours are relevant for humans, these should be taken into account for classification of cyflumetofen for carcinogenicity.

RAC Conclusion

There has been a great deal of discussion presented by the DS and industry representatives. All are good points and are well explained in the supporting documentation provided during public consultation. These points try to answer several questions but ultimately fail to remove the concern that the small but biologically significantly increased incidence of C-cell carcinomas are induced by cyflumetofen treatment.

Given that the evidence of carcinogenicity is restricted to a single experiment and single sex, at one high dose level and thus limited in nature, and taking into account that the potential relevance for humans cannot be ignored, classification of cyflumetofen for carcinogenicity as Carc. 2 (H351: Suspected of causing cancer) is considered appropriate by RAC.

If RAC accepts that the normal pathogenesis of C-cell carcinoma is a progression from hyperplasia to adenoma to C-cell carcinoma and that the precursor lesions (hyperplasia and adenomas) typically have a higher incidence than the final malignant tumours, then it is evident that this model is not supported by the findings in both the Yoshida (2004) and Takahashi (2013) rat lifetime studies. There was no indication of precursor proliferative lesions of the C-cell in shorter duration studies, or in studies with lower doses of cyflumetofen, i.e. no dose response and no time course for development of the pre-neoplastic lesions and malignant tumours. Methodological factors may influence the identification of proliferative lesions and the ability to distinguish them from adenomas, particularly in a small gland such as the rat thyroid (Hardistry, 1985). Adenomas are typically described as regions of hyperplasia greater than five follicles in width. Sectioning of the thyroid is critical for the identification of small tumours such as C-cell adenomas. Industry confirmed the standard method employed was a single random section through the gland. Serial sectioning of this gland has been shown to be a better technique in establishing the true incidence of small tumours and regions of hyperplasia (Thompson & Hunt, 1963). It is possible that such information has been missed in these studies because serial sectioning of the thyroid was not employed.

There was no support for carcinogenicity from studies in other species. The mouse lifetime studies recorded no C-cell lesions at any dose or time point, even though the top dose tested was approximately 5-fold higher than that in the rat lifetime study.

The evidence for a treatment related effect is weak. There is no dose response relationship. There is however, a real signal above background; it is statistically significant at the highest dose tested in a susceptible rat strain. There is therefore sufficient uncertainty for RAC to conclude that **classification as Carc. 2 (H351: Suspected of causing cancer) is warranted**.

4.11   Toxicity for reproduction

Table 102 Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
2-generation reproduction study rat, BrlHan:WIST@Tac(GALAS) 0, 150, 500 or 1500 mg/kg food, equal to 0, 10.4, 34.6 and 100.3 mg/kg bw/d for males and 0, 12.0, 39.7 and 121.6 mg/kg bw/d for females	NOAEL parental: 10 mg/kg bw per day NOAEL developmental: 10 mg/kg bw/d NOAEL reproduction: 10 mg/kg bw/d	LOAEL parental: 34.6 mg/kg bw/day based on increased adrenal weight and hypertrophy of adrenal cortical cells LOAEL developmental: 34.6 mg/kg bw/day based on increased adrenal weight and hypertrophy of adrenal cortical cells LOAEL : 34.6 mg/kg bw/day based on slight delay in sexual development.	[a]Takahashi, 2004
Teratogenicity study rat, WIST@Tac(GALAS) (oral gavage) 0, 50, 250 or 1000 mg/kg bw/d	NOAEL parental: 50 mg/kg bw per day NOAEL developmental: 50 mg/kg bw/d NOAEL teratogenicity: ≥ 1000 mg/kg bw/d	LOAEL maternal: 250 mg/kg bw/day based on increased adrenal weight and vacuolation of adrenal cortical cells LOAEL developmental: 250 mg/kg bw/day based on delayed ossification Teratogenicity: No findings	[a]York, 2001
Teratogenicity study rabbit, Hra:(NZW) (oral gavage) 0, 50, 250 or 1000 mg/kg bw/d	NOAEL parental: 50 mg/kg bw per day NOAEL developmental: 250 mg/kg bw/d NOAEL teratogenicity: ≥ 1000 mg/kg bw/d	LOAEL maternal: 250 mg/kg bw/day based on decreased body weight gain LOAEL developmental: 1000 mg/kg bw/day based on incomplete ossification, hyoid changes and reduced foetal weight. Teratogenicity: No findings	[a]York, 2003a

[a] As summarised in revised DAR_2011_vol3 B6 (September and October 2011) and PRAPeR meeting outcome (EFSA, 2011)

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Two-generation reproduction study in rats (Takahashi, 2004)

reference	:	Takahashi, 2004	exposure	:	F0: 10 weeks before mating until sacrifice F1: during weaning, premating period until sacrifice By diet
type of study	:	2-generation reproduction study	doses	:	: 0, 150, 500, 1500 mg/kg food[1]
year of execution	:	2002-2004	vehicle	:	None
test substance	:	OK-5101; batch 01D1, purity 97.67%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 416
species	:	Rat, BrlHan:WIST@Tac(GALAS)	acceptability	:	Acceptable
group size	:	24/sex/dose (F0 and F1)	**NOAELpar**	:	10 mg/kg bw/d
			NOAELdev		10 mg/kg bw/d
			NOAELrepro		≥ 100 mg/kg bw/d

[1] equivalent to 0, 10.4, 34.6 and 100.3 mg/kg bw/d for males and 0, 12.0, 39.7 and 121.6 mg/kg bw/d for females, based on intake by F0 animals during pre-mating

A two generation reproduction study was performed in accordance with OECD 416. Rats were given cyflumetofen (purity 97.67%) at dietary levels of 0, 150, 500 or 1500 mg/kg food (equivalent to 0, 10.4, 34.6 and 100.3 mg/kg bw/d for males and 0, 12.0, 39.7 and 121.6 mg/kg bw/d for females, based on intake by F0 animals during pre-mating) starting 10 weeks before mating (F0) or at weaning (F1) until scheduled necropsy. Dose levels were based on a range finding study in which rats were dosed at levels up to 3000 mg/kg food, which revealed no effects on fertility, and at 1000 mg/kg food effect on adrenal weights and cortical cell vacuolation in both parental animals and P1 pups.

The results of the main 2-generation study are summarised in **Table 103** to Table 106.

Body weight development, food consumption, clinical observations and parental mortality were not affected by treatment.

Absolute and relative adrenal weight was increased in F0 females at 500 mg/kg food (112 and 112% of control, respectively) and in both sexes at 1500 mg/kg food (112-113 and 112-125%, respectively). The effect in females showed a dose-response relationship. Absolute and relative pituitary weight was increased at 1500 mg/kg food in females (110 and 111% of control, respectively); relative pituitary weight was increased at 1500 mg/kg food in males (110% of control, respectively). Absolute and relative thyroid weight was increased at 1500 mg/kg food in females (both 109% of control). At 1500 mg/kg food an increased relative ovary weight (111% of control) was noted. Further changes in organ weights (e.g. pituitary, adrenals and thyroid weight at 150 mg/kg food; liver at 500 mg/kg food) were not considered toxicologically relevant since changes were neither dose-related nor accompanied by histopathological changes.

Macroscopical examination revealed white coloured adrenal glands in one female at 500 mg/kg food and 5 males and 4 females at 1500 mg/kg food. Enlarged adrenal glands were observed in 5 females at 1500 mg/kg food. An increased incidence of hypertrophy of the zona glomerulosa was noted in females at 500 mg/kg food and both sexes at 1500 mg/kg food with a dose-response relationship in females. Hypertrophy of the zona fasciculata showed an increased incidence in

females at 1500 mg/kg food. An increased incidence of vacuolation of the zona fasciculata was noted in males at 500 mg/kg food, without dose-response.

Absolute and relative adrenal weight was increased at 500 and 1500 mg/kg food in F1 females (115 and 120% of control for absolute and 115 and 121% of control for relative weight) and relative adrenal weight was increased at 1500 mg/kg food in F1 males (112% of control). The toxicological significance of decreased absolute prostate weight at 1500 mg/kg food (87% of control) and increased relative pituitary weight in females at 1500 mg/kg food (109% of control) was not clear. Further changes in organ weights (pituitary and uterus at 150 mg/kg food) were not considered to be toxicologically relevant as no dose-relationship was observed. The incidence of white coloured adrenal glands was significantly increased in both F1 sexes at 1500 mg/kg food and the incidence of enlarged adrenal glands was significantly increased in females at 1500 mg/kg food. An increased incidence of hypertrophy of the zona glomerulosa was noted at 500 mg/kg food and 1500 mg/kg food in both sexes. Hypertrophy of the zona fasciculata showed an increased incidence in females at 1500 mg/kg food. Vacuolation of the zona fasciculata was noted in an increased number of males at 1500 mg/kg food.

Oestrus cycle length and serum hormone concentrations.

The oestrous cycle length was slightly increased in F1 females (105% of control) at 1500 mg/kg food. Serum concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone in F1 males were comparable to controls for all dose groups. Serum concentration of FSH was significantly decreased in females at 500 and 1500 mg/kg food (49% of control) non-dose-dependently. Progesterone was significantly decreased in all female dose groups in a dose-related manner (53-69% of control). 17β-oestradiol was significantly decreased in females at 1500 mg/kg food (66% of control). It should be noted that variations in the control animals are very large for progesterone (factor 3) and 17β-oestradiol (factor 1.8) (for progesterone and 17β-oestradiol all values of the dose groups remained within the range of the control values) and the number of animals investigated (n=10) is not considered sufficient to reliably detect changes in serum hormone levels, and therefore observed changes should carefully be interpreted. At the performing laboratory no historical control data are available on FSH, progesterone or 17β-oestradiol.

In the 1500 mg/kg food group, the oestrus cycle length was prolonged and changes in weights and histopathological findings of the pituitary and ovaries were observed in F1 females. In addition, serum FSH, progesterone and 17beta-estradiol concentration of F1 females at 1500 mg/kg food were significantly lower than controls when measured after weaning of the F2 pups.

Although an increase in oestrus cycle length was noted at 1500 mg/kg food, the maximum oestrus cycle length was 6 days, however, this was only observed in 2 females, 17 females showed an oestrus cycle length of 4 days, and 3 females of 4-5 days and 2 females of 5 days. In the P0 and F1 generation control animals the range of oestrus cycle length was 4-5 days. The mean oestrus cycle length at the high dose is well within the historical control range, the mean oestrus cycle length at the control might be even at the low end of the historical control range. Furthermore, mating behaviour was not affected. Therefore, the increased oestrus cycle length is not considered toxicologically relevant.

Relative to the control, sexual maturation (completion of preputial separation) took 1 day longer in F1 males at 1500 mg/kg food. However, there was no hormonal basis under the delayed sexual development because F1 males in the group did not show any significant alteration neither in serum concentrations of FSH, LH, and testosterone nor weights and histopathological findings of the

reproductive organs. Furthermore, observed duration was well within the historical control range (see Table 108).

In the 500 mg/kg food group, sexual development of F1 females (completion of vaginal opening) took 1.2 day longer when compared to controls. However, observed duration was well within the historical control range, and the reproductive performance after sexual maturation was normal, and no significant changes were noted in histopathological observation of the reproductive organs and the serum 17beta-estradiol concentration (see Table 109).

However, the experts in the PRAPeR meeting indicated that in Table 105 and Table 106, slight effects on hormonal levels in females seems to be correlated with delayed sexual maturation; in the presence of statistical significance, dose response, effects in both sexes, presumed effect on fatty metabolism (unclear mode of action), these effects could possibly be linked to cyflumetofen and should be part of the NOAEL determination. A NOAEL can be identified at the low dose level. The historical control data only indicates that the study controls are unusually low (which will determine the statistical significance in the study) but it was considered that they are not excluding an effect of the substance.

No treatment-related changes were detected in litter size, post-implantation loss, live birth index, viability index, lactation index, sex ratio, clinical signs or pup weight of the F0 offspring (F1 pups) (see **Table 103** and
Table 104). Absolute and relative adrenal weight was increased at 500 and 1500 mg/kg food in both sexes with a dose-related response (113-117% and 120-127% of control at 500 and 1500 mg/kg food, respectively).
 No macroscopical changes were noted at 150 and 500 mg/kg food groups. At 1500 mg/kg food, males showed white color of the adrenals, and females showed enlargement and white color of adrenals. Histopathological examination showed an increased incidence of hypertrophy of the zona glomerulosa in males at 500 mg/kg food and in both sexes at 1500 mg/kg food. An increased incidence of hypertrophy of the zona fasciculata was noted at 500 and 1500 mg/kg food in both sexes with a dose-related response.

No treatment-related changes were detected in litter size, post-implantation loss, live birth index, viability index, lactation index, sex ratio or clinical signs of the F2 pups (see Table 103 and
Table 104). Pup weights were significantly decreased on day 7-21 of lactation at 1500 mg/kg food in both sexes (91-94% of control). The relative anogenital distance of the F2 males was significantly increased at 150 and 1500 mg/kg bw/d. Since this was an increase indicating no delayed sexual development and no dose-related response was noted, this effect is not considered to be toxicologically significant. Absolute and relative adrenal weight was increased at 500 and 1500 mg/kg food in males (113% and 111-123% of control) and females (108-116% and 110-126% of control) in a dose-related manner. Absolute thymus weight was decreased in females at all dose groups and relative thymus weight was decreased in females at 500 mg/kg food. Since the absolute thymus weight of the control group was rather high, no histopathological effects were seen and changes were slight, changes in thymus weight are not considered to be toxicologically relevant. The increased relative brain weight in both F2 sexes and decreased absolute spleen and thymus weight in F2 females at 1500 mg/kg food were considered to be related to reduced body weight. No macroscopical changes were noted at 150 and 500 mg/kg food groups. At 1500 mg/kg food, males showed white color of the adrenals, and females showed enlargement and white color of adrenals. . Histopathological examination of the adrenal glands showed an increased incidence of hypertrophy of the zona fasciculata and glomerulosa at 500 mg/kg food in F2 males and at 1500 mg/kg food in both F2 sexes.

At 150 mg/kg food, no alterations were found in reproductive parameters of the parental males and females, with the exception of a slight decrease in serum progesterone in F1 females. As no further changes were seen, the slight decrease in serum progesterone is not considered toxicologically relevant. The agreed reproductive NOAEL is 10 mg/kg bw/d based on delays in sexual development seen at the two higher dose levels, statistically significant above the controls. Considering the absence of effects on the usual reproductive parameters, this was considered as a conservative approach by the experts in the PRAPeR meeting.

Table 103 Summary table (Takahashi, 2004)

Dose (mg/kg food)	0		150		500		1500		dr
	m	f	m	f	m	f	m	f	
F0 animals									
Mortality	None								
Clinical signs	No treatment-related findings								
Body weight gain	No treatment-related findings								
Food consumption	No treatment-related findings								
Mating/fertility/gestation	No treatment-related findings								
Oestrus cycle	No treatment-related findings								
Sperm evaluation	No treatment-related findings								
Organ weight - adrenals - pituitary - ovaries - thyroids - liver			ica,r			ica,r	ica,r icr	ica,r ica,r icr ia, icr	f
Pathology									
Macroscopy - adrenal gland: white colour - adrenal gland: enlarged	0/24 0/24	0/24 0/24	0/24 0/24	0/24 0/24	0/24 0/24	1/24 1/24	5/24 0/24	4/24 5/24	
Microscopy adrenal: -hypertrophy, zona glomerulosa -hypertrophy, zona fasciculata -vacuolation, zona fasciculata	4/24 0/24 1/24	4/24 6/24 0/24	4/24 0/24 6/24	4/24 7/24 0/24	4/24 0/24 9/24	13/24 10/24 0/24	14/24 0/24 4/24	19/24 18/24 0/24	f
F1 pups									
Litter size	No treatment-related findings								
Post implantation loss (%)	No treatment-related findings								
Live birth index	No treatment-related findings								
Viability index	No treatment-related findings								

Dose (mg/kg food)	0		150		500		1500		dr
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	
Lactation index	No treatment-related findings								
Sex ratio	No treatment-related findings								
Clinical signs	No treatment-related findings								
Body weight	No treatment-related findings								
Organ weights - adrenals					ica,r	ica,r	ica,r	ica,r	m/f
Pathology									
<u>Macroscopy</u>	No treatment-related findings								
<u>Microscopy</u> adrenal: -hypertrophy, zona glomerulosa -hypertrophy, zona fasciculata	0/23 5/23	0/23 3/23	1/22 2/22	1/22 3/22	6/23 8/23	3/23 7/23	11/23 21/23	13/23 17/23	m m/f
F1 animals									
Mortality	None								
Clinical signs	No treatment-related findings								
Body weight gain	No treatment-related findings								
Food consumption	No treatment-related findings								
Sexual maturation						ic	ic	ic	
Mating/fertility/gestation	No treatment-related findings								
Oestrus cycle								ic	
Sperm evaluation	No treatment-related findings								
Organ weight - adrenals - prostate - pituitary - uterus				ica ica		ica,r	icr dca	ica,r icr	
Pathology									
<u>Macroscopy</u> - adrenal: white colour - adrenal: enlarged	0/24 0/24	0/24 0/24	0/24 0/24	0/24 0/24	0/24 0/24	2/24 1/24	9/24 0/24	15/24 9/24	
<u>Microscopy</u> adrenal: -hypertrophy, zona glomerulosa -hypertrophy, zona fasciculata -vacuolation, zona fasciculata	4/24 0/24 3/24	1/24 6/24 0/24	5/24 0/24 4/24	5/24 8/24 0/24	9/24 0/24 3/24	7/24 7/24 0/24	11/24 0/24 8/24	21/24 13/24 0/24	m/f

Dose (mg/kg food)	0		150		500		1500		dr
	m	f	m	f	m	f	m	f	
prostate, mononuclear cellular infiltration	3/24		2/24		2/24		8/24		
ovary, interstitial vacuolation		2/24		2/24		3/24		14/24	
Serum hormone analysis									
- FSH						dc		dc	
- progesterone				dc		dc		dc	f
- 17β-oestradiol								dc	
F₂ pups									
Litter size	no treatment-related findings								
Post implantation loss	no treatment-related findings								
Live birth index	no treatment-related findings								
Viability index	no treatment-related findings								
Lactation index	no treatment-related findings								
Sex ratio	no treatment-related findings								
Clinical signs	no treatment-related findings								
Body weight day 7-21 post-partum							dc	dc	
Anogenital distance			icr				icr		
Organ weights									
- adrenals					ica,r	ia, icr	ica,r	ica,r	m/f
- spleen								dca	
- thymus				dca		dca,r		dca	
- brain							icr	icr	
Pathology									
Macroscopy	No treatment-related findings								
Microscopy adrenal:									
-hypertrophy, zona glomerulosa	3/23	2/23	2/23	3/23	6/21	0/21	14/24	10/23	
-hypertrophy, zona fasciculata	1/23	4/23	0/23	4/23	8/21	4/21	22/24	16/23	m

dr dose related

dc/ic statistically significantly decreased/increased compared to the controls

d/i decreased/increased, but not statistically significantly compared to the controls

a/r absolute/relative organ weight

+ present in one/a few animals

++ present in most/all animals

1 in week 3 – 5

2 in week 1- 4

3 throughout the pre-breeding period

4 A subsequent 2-generation study was performed at concentrations of 7.5, 15, and 20 mg/kg food in order to further assess the equivocal parental body weight effects noted at 20 mg/kg food. The effect was not reproduced in the

subsequent study. In addition the effects on spleen weight in the pups of the 20 mg/kg group were not reproduced. Therefore, the reductions in mean body weight at 20 mg/kg food were not attributed to test substance administration.

Table 104 Reproduction and gestational parameters of female rats treated with cyflumetofen (Takahashi, 2004)

Parental generation	F0				F$_1$			
Dose [mg/kg]	0	150	500	1500	0	150	500	1500
Animals per dose	24	24	24	24	24	24	24	24
Female fertility								
- placed with males	24	24	24	24	24	24	24	24
- mated [n]	24	24	24	24	24	24	24	24
- mating index [%]	100	100	100	100	100	100	100	100
-number of days until mating	1.0	1.1	1.0	1.0	1.0	1.2	1.0	1.1
- pregnant [n]	24	22	23	23	23	23	21	24
- **Fertility index** [%]	100.0	91.7	95.8	100.0	95.8	95.8	87.5	100.0
Estrous cycle length [days]	4.0	4.0	4.0	4.1	4.0	4.0	4.0	4.2*
Pre coital interval [days]								
Duration of gestation [days]	22.2	22.1	22.2	22.2	22.2	22.1	22.0	22.1
Implantation sites, total [n]	12.2	12.5	13.0	12.6	11.0	12.2	11.9	12.3
Post implantation loss [n]	12	18	19	16	14	21	14	19
- implantation loss per dam [n]	0.50	0.82	0.83	0.70	0.61	0.91	0.67	0.79
implantation loss per litter [mean %]	5.20	7.69	7.38	7.17	8.97	10.07	6.54	7.84
Females with liveborn								
- **Gestation index** [%]	100	100	100	100	100	100	100	100
- dams with stillborn pups [n]	2	0	2	0	0	0	0	1
- dams with all stillborn [n]	0	0	0	0	0	0	0	0
Pups delivered [n]	281	256	281	274	239	260	235	275
- per dam [mean n]	11.7	11.6	12.2	11.9	10.4	11.3	11.2	11.5
- liveborn [n]	278	256	279	274	239	260	235	273
- stillborn [n]	3	0	2	0	0	0	0	2
- **Live birth index** [%]	99.1	100	99.4	100	100	100	100	99.2

* p ≤ 0.05; ** p ≤ 0.01 (Dunnet-test two sided or Fisher's exact test one sided)

Values may not calculate exactly due to rounding of values

Table 105 Group mean serum hormone concentrations in F1 parental rats (Takahashi, 2004)

Sex	Dose (ppm)	No. of Animals	LH (ng/mL)		FSH (ng/mL)		Testosterone (ng/mL)		Progesterone (ng/mL)		17B-Estradiol (pg/mL)	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Male	0	10	1.48	0.29	9.63	1.67	1.20	0.92	-	-	-	-
	150	10	1.44	0.23	9.97	1.44	0.81	0.55	-	-	-	-
	500	10	1.28	0.31	8.39	1.97	0.53	0.47	-	-	-	-
	1500	10	1.57	0.25	10.06	1.46	0.80	0.89	-	-	-	-
Female	0	10	1.48	0.16	7.58	0.87	-	-	15.7	4.1	19.3	4.9
	150	10	1.49	0.15	9.32	1.06	-	-	10.9*	4.7	18.6	6.1
	500	10	1.61	0.17	3.70*	2.09	-	-	9.9**	3.5	17.4	4.8
	1500	10	1.54	0.30	3.69*	1.66	-	-	8.3**	2.4	12.7*	4.9

* $p \leq 0.05$; ** $p \leq 0.01$

Table 106 Sexual development in F1 parental rats (cyflumetofen) (Takahashi, 2004)

Dose (ppm)	Preputial separation in MALE		Completion of vaginal opening in FEMALE	
	Days of age	Body Weight (g)	Days of age	Body Weight (g)
0	40.9	178	29.5	92
150	40.5	179	30.0	95
500	41.2	179	30.7*	96
1500	41.9*	182	31.0**	95

* $p \leq 0.05$; ** $p \leq 0.01$

According to the historical control data, mean oestrus cycle lengths with standard deviations (S.D.) of control females ranged from 4.0±0.1 to 4.1±0.4 in the parental (P) generation and that in the F1 generation was between 4.0±0.1 and 4.3±0.5 (see
Table 107). This reveals that the values in the high-dose group of cyflumetofen study, 4.1±0.2 and 4.2±0.4 in the F0 and F1 generations, respectively, are within the normal range of this stock of rats, suggesting that the minor alterations in oestrus cycle length noted in the high-dose group are not adverse, although the difference from concurrent controls was statistically significant only in the F1 generation.

Table 107 Historical control data on oestrus cycle length in female Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats

Study no.	Reported year	Oestrus cycle length (days, mean $\pm$ S.D.)	
		Parental (F0) generation	F1 generation
1	2004	4.1 $\pm$ 0.3	4.2 $\pm$ 0.3
2	2005	4.0 $\pm$ 0.1	4.2 $\pm$ 0.4
3	2005	4.0 $\pm$ 0.2	4.1 $\pm$ 0.3
4	2007	4.1 $\pm$ 0.2	4.0 $\pm$ 0.1
5	2007	4.0 $\pm$ 0.2	4.3 $\pm$ 0.5
6	2008	4.1 $\pm$ 0.2	4.3 $\pm$ 0.5
7	2008	4.1 $\pm$ 0.4	4.1 $\pm$ 0.2
8	2009	4.0 $\pm$ 0.2	4.0 $\pm$ 0.1
9	2009	4.0 $\pm$ 0.2	4.2 $\pm$ 0.4

Sexual developments of F1 offspring in the middle- and/or high-dose groups also seemed to be slightly delayed in the 2-generation study, in which mean days of age at completion of preputial separation in the high-dose group (41.9±1.5), as well as of vaginal opening in the middle- and high-dose groups (30.7±2.1 and 31.0±1.9, respectively) statistically significantly but slightly exceeded the control values (40.9±1.5 in males and 29.5±2.2 in females). As shown in
Table 108 and Table 109, however, these alterations are considered not to be an indication of delay in sexual maturation because they are within the historical control range (mean days of age at preputial separation, 40.7±1.5 – 42.5±2.1; mean days of age at vaginal opening, 28.2±1.3 - 31.9±2.4) and because body weights of these animals at the day of completion did not differ significantly from the concurrent control value even in the high-dose group.

Table 108 Historical control data on preputial separation of F1 males in Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats

Study no.	Reported year	Completion of preputial separation (mean $\pm$ S.D.)	
		Days of age	Body weights (g) at completion
1	2004	41.3 $\pm$ 2.0	176 $\pm$ 16
2	2005	40.7 $\pm$ 1.5	179 $\pm$ 16
3	2005	42.3 $\pm$ 2.0	182 $\pm$ 10
4	2007	41.2 $\pm$ 1.8	175 $\pm$ 14
5	2007	41.0 $\pm$ 1.4	184 $\pm$ 14
6	2008	42.3 $\pm$ 1.4	188 $\pm$ 13
7	2008	42.3 $\pm$ 2.4	182 $\pm$ 19
8	2009	42.3 $\pm$ 1.9	181 $\pm$ 10
9	2009	42.5 $\pm$ 2.1	189 $\pm$ 17

Table 109 Historical control data on vaginal opening of F1 females in Wistar Hannover (BrlHan:WIST@Jcl[GALAS]) rats

Study no.	Reported year	Completion of vaginal opening (mean $\pm$ S.D.)	
		Days of age	Body weights (g) at completion
1	2004	31.8 $\pm$ 2.0	102 $\pm$ 10
2	2005	30.8 $\pm$ 2.4	97 $\pm$ 12
3	2005	31.0 $\pm$ 2.3	97 $\pm$ 11
4	2007	31.6 $\pm$ 1.8	97 $\pm$ 10
5	2007	28.2 $\pm$ 1.3	84 $\pm$ 7
6	2008	30.3 $\pm$ 1.5	92 $\pm$ 10
7	2008	31.9 $\pm$ 2.4	101 $\pm$ 11
8	2009	29.7 $\pm$ 2.5	90 $\pm$ 13

Study no.	Reported year	Completion of vaginal opening (mean ± S.D.)	
		Days of age	Body weights (g) at completion
9	2009	31.9 ± 2.3	102 ± 10

In Table 110 a summary of all findings in reproductive organs is given, in order to have a complete overview of all effects on reproductive organs/functions in the repeated dose studies including species and dose levels.

In the 2-generation reproductive study, at 34.6 mg/kg bw/day, no alterations were found in reproductive parameters of the parental males and females, with the exception of a slight decrease in serum progesterone in F1 females. As no further changes were seen, the slight decrease in serum progesterone is not considered toxicologically relevant. The agreed reproductive NOAEL is 10 mg/kg bw/d based on delays in sexual development seen at the two higher dose levels, statistically significant above the controls.

In the repeated dose toxicity study in rats and dogs, vacuolation of the ovary and testes were found at dose levels of 69 mg/kg bw/day and above.

Table 111 Overview of all effects on reproductive organs/functions in repeated dose studies

Study	NOAEL (mg/kg bw/day)	LOAEL (mg/kg bw/day)	Finding	Reference
14-day oral dietary toxicity study, OECD 407, rat	< 105 (females)	105 (females)	Vacuolisation of interstitial gland cells in ovary Vacuolation of corpora lutea in ovary at 1000 mg/kg bw/day	Sakia, 2001
28-day oral (dietary) toxicity study OECD 407, rat	132 (females)	351 (females)	Increased ovary weight, vacuolation of luteal cells of corpora lutea in the ovary	Buesen, 2010
28 day oral (dietary) toxicity study OECD 407, rat	40.8 (females)	79.8 (females)	Vacuolation of interstitial cells in ovary	Yoshida, 2004a
13 week oral (dietary) toxicity study OEC 408, rat	62.8 (females)	193 (females)	Vacuolation, interstitial gland cells in ovary	Yoshida, 2004c
13 week oral (gelatin capsule) toxicity study OECD 409, dog	300 (males)	1000 (males)	Increased absolute and relative testis weight	Nagashima, 2003b
Long term (12 months) toxicity study in rats OECD 452	23.3 (females)	69.2 (females)	Vacuolation of interstitial gland cells in ovaries	Yoshida, 2004e
Long term (1 year) oral toxicity in rats (MTD) OECD 452,	< 250 (males) < 319 (females)	250 (males) 319 (females)	Hyperplasia of the interstitial cells of the testes. Vacuolation of the interstitial gland cells of the ovary.	Yoshida, 2012

4.11.1.2 Human information

No data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Teratogenicity study in rats (York, 2001)

reference	:	York 2001	exposure	:	GD6-19 (oral gavage)
type of study	:	Prenatal developmental toxicity study	doses	:	: 0, 50, 250, 1000 mg/kg bw/d
year of execution	:	2001	vehicle	:	aqueous 5% arabic gum with 0.4% Tween®80
test substance	:	OK-5101; batch 01D1, purity 97.67%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 414
species	:	Rat, BrlHan:WIST@Tac(GALAS)	acceptability	:	Acceptable
group size	:	25 females/dose	**NOAELmat**	:	50 mg/kg bw/d
			NOAELdev		50 mg/kg bw/d
			NOAELterato		≥ 1000 mg/kg bw/d

A teratogenicity study was performed in rats in accordance with OECD 414. Female rats were mated and administered 0, 50, 250 or 1000 mg/kg bw/d cyflumetofen by gavage (purity 97.67%) from day 6-19 of gestation. The results are summarised in Table 112. Detailed information on body weights and organs weights are summarized in Table 113 and

Table 114.

No mortality nor clinical signs were observed throughout the study. Body weight gain during day 6-20 of gestation was decreased (77% of control) at 1000 mg/kg bw/d. Body weights and food consumption were comparable among the 0, 50, 250 and 1000 mg/kg bw/day dose groups. Uterus weights were similar to those of control animals. Absolute and relative adrenal weight was increased for the left adrenal at 250 and 1000 mg/kg bw/d (115 and 133% of control for absolute, and 112 and 134% of control for relative weight) and for the right adrenal at 1000 mg/kg bw/d (133 and 135% of control for absolute and relative weight, respectively). No gross necropsy observations were made at Caesarean section. Microscopic examination of the adrenals revealed an increased incidence of slight diffuse hypertrophy of cortical cells at 1000 mg/kg bw/d and slight bilateral diffuse cytoplasmic vacuolation of cortical cells at 250 and 1000 mg/kg bw/d.

No treatment-related effect was found on the number of resorptions, the number of live foetuses, foetal weight or sex ratio (see figures in next Table). No external malformations or variations were observed. No treatment-related visceral (soft tissue) malformations were noted in this study. The only malformation consisted of microphthalmia of the right eye occurred in one fetus in the 50 mg/kg/day dosage group. No additional alterations occurred in this fetus. Folded retina of the left eye, an alteration usually attributed to processing, occurred in one fetus in the 50 mg/kg/day dosage group. Skeletal examination showed a delayed ossification at 250 and 1000 mg/kg bw/d as shown by incompletely ossified sternal centra. The higher foetal incidence of wavy ribs observed at 1000 mg/kg bw/d was not considered treatment-related, because no effect was observed on the litter incidence.

The maternal NOAEL is set at 50 mg/kg bw/d based on increased adrenal weight and increased incidence of vacuolation of adrenal cortical cells. The NOAEL for developmental effects is set at 50 mg/kg bw/d based on delayed ossification. Since no irreversible structural effects were reported, the NOAEL for teratogenic effects is set at ≥ 1000 mg/kg bw/d.

Table 112 Summary table (York, 2001)

Dose (mg/kg bw/d)	0	50	250	1000	dr
Maternal effects					
Mortality (n=25)	0	0	0	0	
Clinical signs		No treatment-related findings			
Pregnant animals (n=25)	24	23	23	25	
Body weight gain					
gestation day 6-20				dc	
Food consumption		No treatment-related findings			
Organ weights - adrenal left - adrenal right			$ic^{a,r}$	$ic^{a,r}$ $ic^{a,r}$	f
Pathology					
macroscopy		No treatment-related findings			
microscopy adrenal cortical cells: -diffuse hypertrophy -diffuse bilateral cytoplasmic vacuolation	0/25 0/25	0/25 0/25	0/25 2/25	14/25** 24/25**	
Litter response					
Number of dams examined	24	23	23	25	
Corpora lutea/dam	15.8	15.2	15.3	15.0	
Implantations/dam	13.5	13.1	14.2	12.9	
Number of resorptions / dam - early - late	32 0	14 1	12 0	17 1	
Dams with live foetuses	24	23	23	25	
Live foetuses/dam	12.2	12.5	13.7	12.2	
Foetal weight	3.34	3.22	3.26	3.22	
Sex ratio		No treatment-related findings			

Dose (mg/kg bw/d)	0	50	250	1000	dr
Examination of the foetuses					
External observations	No treatment-related findings				
Skeletal findings Foetal incidence: -wavy ribs	6	5	9	16**	
Litter incidence: - incompletely ossified sternal centra	4	2	9**	9*	
Visceral findings	No treatment-related findings				

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative organ weight
*   significantly different from control ($p<0.05$)
**   significantly different from control ($p<0.01$)

Table 113 Mean gravid uterus weights and net body weight change of pregnant rats administered cyflumetofen during Days 6 to 19 of gestation (York, 2001)

Dose level [mg/kg bw/d]	0	50	250	1000
Gravid uterus (g)	63.899 ± 11.685	65.367 ± 15.422	72.230 ± 6.857	65.463 ± 11.894
Carcass (g)	344.7 ± 21.9	350.5 ± 29.4	355.6 ± 17.8	343.5 ± 23.7
Net weight change from Day 6 (g)	27.1 ± 8.8	27.2 ± 9.9	26.2 ± 7.1	$20.9 \pm 8.4*$

* $p \leq 0.05$

Table 114 Mean absolute and relative liver, kidney and adrenal weights of pregnant rats administered cyflumetofen during Days 6 to 19 of gestation (York, 2001)

	Dose [mg/kg bw/day]	Absolute weight [g]	% of Control	Relative weight [% of b.w.]	% of Control
Terminal bodyweight [g]	0	344.7	100	-	
	50	350.5	102	-	
	250	355.6	103	-	
	1000	343.5	100	-	
Liver [g]	0	14.98	100	4.348	100
	50	14.53	97	**4.136***	95
	250	15.24	102	4.285	99
	1000	15.23	102	4.450	102
Kidney (Left) [g]	0	1.02	100	0.294	100
	50	1.01	99	0.288	98
	250	0.97	95	0.273	93
	1000	0.97	95	0.283	96
Kidney (Right) [g]	0	1.05	100	0.303	100
	50	1.04	99	0.296	98
	250	0.99	94	**0.279****	92
	1000	1.01	96	0.296	98
Adrenal (Left) [g]	0	0.048	100	13.891	100

	Dose [mg/kg bw/day]	Absolute weight [g]	% of Control	Relative weight [% of b.w.]	% of Control
	50	0.051	106	14.662	106
	250	**0.055***	115	**15.504***	112
	1000	**0.064****	133	**18.575****	134
Adrenal (Right) [g]	0	0.045	100	13.011	100
	50	0.047	104	13.502	104
	250	0.049	109	13.952	107
	1000	**0.060****	133	**17.606****	135

* p ≤ 0.05; ** p ≤ 0.01 (Kruskal-Wallis and Wilcoxon-test, two sided)

Values may not calculate exactly due to rounding of figures

Teratogenicity study in rabbits (York 2003)

reference	:	York 2003	exposure	:	GD6-28 (oral gavage)
type of study	:	Prenatal developmental toxicity study	doses	:	: 0, 50, 250, 1000 mg/kg bw/d
year of execution	:	2002	vehicle	:	aqueous 5% arabic gum with 0.4% Tween®80
test substance	:	OK-5101; batch 01D1, purity 97.67%	GLP statement	:	Yes
route	:	Oral	guideline	:	According to OECD 414
species	:	Rabbits, Hra:(NZW)	acceptability	:	Acceptable
group size	:	25 females/dose	**NOAELmat**	:	50 mg/kg bw/d
			NOAELdev		50 mg/kg bw/d
			NOAELterato		≥ 1000 mg/kg bw/d

A developmental toxicity study was performed in rabbits in accordance with OECD 414. Female rabbits were mated and administered 0, 50, 250 or 1000 mg/kg bw/d cyflumetofen by gavage (purity 97.67%) during days 6-28 of gestation. The results are summarised in Table 115.

No treatment-related mortality was observed. One dam at 250 mg/kg bw/d was found dead on day 8 shortly after administration. She had shown no adverse clinical signs and body weight gain and food consumption were normal. Necropsy revealed a perforation in the right diaphragmatic lobe of the lungs with red fluid in the thoracic cavity and pale and spongy lung lobes. Another dam at 250 mg/kg bw/d showed clonic convulsions (once only) and red substance in cage pan determined to be blood from the digestive tract. The dam lost 1 kg during day 6-29 and food consumption was severely reduced. Although the dam survived till scheduled necropsy, values for this abnormal dam and litter (completely resorbed) were excluded from the study. No clinical signs were observed throughout the study. Body weight and body weight gain were similar to control values during the study, except for a statistically non-significant decrease in body weight gain during day 6-29 of gestation at 250 and 1000 mg/kg bw/d (76 and 68% of control, respectively). This change in body weight gain was considered to be due to decreased food consumption and observed changes in foetal and placental weights. Gravid uterine weight was decreased at 250 and 1000 mg/kg bw/d (both 95% of control), but not statistically significant. Food consumption was decreased during day 18-21 of gestation at 250 and 1000 mg/kg bw/d (93 and 78% of control, respectively). Absolute liver weight was decreased at 250 and 1000 mg/kg bw/d (both 92% of control). No gross necropsy observations were made at Cesarean section.

No treatment-related effect was found on the number of resorptions, the number of live foetuses or sex ratio (see Table 115). Foetal weight was significantly decreased at 1000 mg/kg bw/d for males and females separately (88 and 90% of control, respectively) and combined (88% of control). The mean placental weight of males and females together was also significantly decreased at 1000

mg/kg bw/d (88% of control). External and visceral examination of the foetuses revealed no treatment-related effects. Skeletal examination showed a delayed development at 1000 mg/kg bw/d as shown by incompletely ossified sternal centra and one or both alae of the hyoid were angulated.

The maternal NOAEL is set at 50 mg/kg bw/d based on decreased body weight gain. The adrenals were not examined microscopically (not a requirement), hence it was unclear whether the adrenal effects observed in the rats would also occur in rabbits. The developmental NOAEL is set at 250 mg/kg bw/d based on incomplete ossification, hyoid changes and reduced foetal weight at 1000 mg/kg bw/day. Since no irreversible structural effects were reported, the NOAEL for teratogenic effects is set at $\geq$ 1000 mg/kg bw/d (see also Table 116).

Table 115 Summary table (York, 2003)

Dose (mg/kg bw/d)	0	50	250	1000	dr
Maternal effects					
Mortality (n=25)	0	0	2	0	
Clinical signs	No treatment-related findings				
Pregnant animals (n=25)	24	24	23	25	
Body weight gain - day 6-29			d	d	
Food consumption day 18-21 of gestation			d	dc	
Gravid uterine weight			d	d	
Organ weights - liver			d	d	
Pathology					
Macroscopy	No treatment-related findings				
Litter response					
Number of dams examined	24	24	22	25	
Corpora lutea/dam	9.4	9.5	9.4	10.0	
Implantations/dam	8.4	8.4	8.4	9.2	
Number of resorptions/dam - early	0.2	0.2	0.1	0.1	
- late	0.7	0.1	0.2	0.4	
Dams with live foetuses	24	24	22	25	
Live foetuses/dam	7.6	8.2	8.1	8.7	
Foetal weight					

Dose (mg/kg bw/d)	0	50	250	1000	dr
- male	47.60	46.56	45.94	41.87**	
- female	45.09	44.36	43.63	40.55**	
Placental weight	7.47	7.00	7.29	6.59*	
Sex ratio	No treatment-related findings				
Examination of the foetuses					
External observations	No treatment-related findings				
Skeletal findings Foetal incidence: - incompletely ossified sternal centra	0	0	1	16**	
Litter incidence: - incompletely ossified sternal centra	0	0	1	8**	
- hyoid, angulated ala	2	2	2	10**	
Visceral findings	No treatment-related findings				

dr dose related
dc/ic statistically significantly decreased/increased compared to the controls
d/i decreased/increased, but not statistically significantly compared to the controls
a/r absolute/relative organ weight
*   significantly different from control (p<0.05)
** significantly different from control (p<0.01)

Table 116 Mean absolute and relative organ weights of pregnant rabbits administered cyflumetofen during Days 6 to 28 of gestation (York, 2003)

	Dose [mg/kg bw./day]	Absolute weight [g]	% of Control	Relative weight [% of b.w.]	% of Control
Terminal weight [g]	0	4244.9	100	-	
	50	4254.8	100	-	
	250	4080.7	96	-	
	1000	4133.7	97	-	
Liver [g]	0	100.8	100	2.374	100
	50	99.6	99	2.331	98
	250	92.9	92	2.268	96
	1000	92.4	92	2.235	94
Kidney (Left) [g]	0	9.2	100	0.218	100
	50	9.1	99	0.212	97
	250	9.1	99	0.222	102
	1000	8.9	97	0.215	99
Kidney (Right) [g]	0	9.0	100	0.213	100
	50	8.9	99	0.208	98
	250	9.0	100	0.220	103
	1000	8.7	97	0.212	100
Adrenal (Left) [g]	0	0.210	100	0.001	-
	50	0.211	100	0.002	-
	250	0.204	97	0.002	-
	1000	0.185	88	0.000	-
Adrenal (Right) [g]	0	0.175	100	0.000	-

	Dose [mg/kg bw./day]	Absolute weight [g]	% of Control	Relative weight [% of b.w.]	% of Control
	50	0.198	113	0.001	-
	250	0.174	99	0.001	-
	1000	0.164	94	0.000	-

4.11.2.2 Human information

No data.

4.11.3 Other relevant information

Endocrine studies (see 4.12.1.3 Specific investigations)

4.11.4 Summary and discussion of reproductive toxicity

In an oral 2-generation reproduction toxicity study in rats, the NOAELs for parental, developmental and reproduction toxicity were set at 10 mg/kg bw/day. At the dose level of 34.6 mg/kg bw/day (LOAEL) both parental and developmental effects included increased adrenal weight and hypertrophy of adrenal cortical cells. At this dose level of 34.6 mg/kg bw/day the only limited effect possibly related to reproduction observed was a slight delay in sexual development (within the historical control range for the rat strain used) and slight effects on hormonal levels in females that – even though statistically significant – are of debatable relevance based on low animal numbers and the absence of historical control data. Considering the absence of effects on the usual reproductive parameters, 10 mg/kg bw/day is considered as a conservative NOAEL for reproduction by the experts in the PRAPeR meeting (EFSA, 2011).

In the repeated dose toxicity study in rats and dogs, vacuolation of the ovary and testes were found at dose levels of 69 mg/kg bw/day and above.

In a teratogenicity study in rats, a NOAEL for maternal toxicity of 50 mg/kg bw/day was derived, based on increased adrenal weight and vacuolation of adrenal cortical cells at 250 mg/kg bw/day (LOAEL) and above. The NOAEL for developmental toxicity was set 50 mg/kg bw/day, based on delayed ossification (incomplete ossified sternal centra) at 250 mg/kg bw/day (LOAEL) and above, a minor reversible variona that is considered transient in nature. No teratogenicity was observed.

In a teratogenicity study in rabbits a NOAEL for maternal toxicity of 50 mg/kg bw/day was derived, based on decreased body weight gain at 250 mg/kg bw/day (LOAEL) and above. The decreased body weight gain was considered to be due to decreased food consumption. The NOAEL for developmental toxicity was set at 250 mg/kg bw/day, based on incomplete ossification, hyoid changes and reduced foetal weight at 1000 mg/kg bw/day. No teratogenic effects were observed.

4.11.5 Comparison with criteria

Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).

- *Category 1A*: Known human reproductive toxicant. The classification of a substance in this Category 1A is largely based on evidence from humans.
- *Category 1B*: Presumed human reproductive toxicant. The classification of a substance in this Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Fertiliy effects

In the oral 2-generation study in rats, the only reproductive effects observed were a slight but statistically significant and dose related delay in male and female sexual maturation within the historical control range for the rat strain used, a statistically significant prolongation of the oestrous cycle length but within the historical control range, an increase in ovary interstitial vacuolation and effects on the serum levels of FSH, progesterone and 17β-oestradiol which are of debatable relevance due to the low number of animals tested. These effecs were seen at a dose level of 500 mg/kg bw/day and above. The effect on the ovary was also observed in repeated dose toxicity studies (at dose levels of 69 mg/kg bw/day and above). However, no significant effects on sexual function and fertility were observed in the rat. The effect on the ovary is most likely a comparable effect as in the adrenals and indicate accumulation of cholesterol and its ester. Effects on adrenals were seen in repeated dose studies in both males and females at dose levels of 49.5 mg/kg bw/day and above and in the 2-generation reproduction study at 34.6 mg/kg bw/day and above. The effect on the sexual maturation is only small and transient and within the historical control range. Therefore, this effect in itself does not justify classification. Also, the effect on the oestrous cycle was small and within the historical control range.

Overall, the observed effects have doubtful toxicological significance and only indicate an effect possibly related to changes in steroidogenesis. Classification for effects on fertility is not required under the EU CLP-regulation.

Developmental toxicity

In the rat 2-generation study, no developmental effects were observed.

In the developmental toxicity study in rats, incomplete ossification was seen at a dose level of 250 mg/kg bw/day, at which maternal toxicity was observed (increased adrenal weight and increased incidence of vacuolation of adrenal cortical cells). In the developmental toxicity study in rabbits, delayed ossification was seen at a dose level of 1000 mg/kg bw/day, at which maternal toxicity was

observed (decreased food consumption and body weight gain). In both the developmental toxicity studies in rats and rabbits, no irreversible structural effects were reported. Classification for effects on development is not required under the EU CLP-regulation.

4.11.6   Conclusions on classification and labelling

No classification is needed.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

In the CLH report, the DS summarised three oral, GLP compliant, reproductive toxicity studies; an OECD TG 416 two generation reproductive toxicity study in rats and two OECD TG 414 teratogenicity studies, one in rats and the other in NZW rabbits.

According to the DS, in the two generation study, the only effects that may be related to reproduction were a slight (but statistically significant and dose-dependent) delay in sexual maturation in males and females (which was within the historical control range for the rat strain used), statistically significant prolongation of the oestrous cycle length (but within the historical control range), an increase in ovary interstitial vacuolation (consistent with the RDT studies) and effects on the serum levels of FSH, progesterone and 17β-estradiol in females which were statistically significant but were of reduced statistical power due to the small sample size based on low animal numbers (no HCD were available). The DS considered that these findings were of doubtful toxicological significance and therefore proposed no classification for effects on fertility.

In the developmental toxicity studies, incomplete ossification was seen at a dose level of 250 mg/kg bw/day in rats and delayed ossification was seen at a dose level of 1000 mg/kg bw/day in rabbits. In both species some evidence of maternal toxicity was observed at these doses; increased adrenal weight and increased incidence of vacuolation of adrenal cortical cells in rats and decreased food consumption and body weight gain in rabbits. In neither species were irreversible structural effects reported. The DS proposed no classification for developmental effects.

Comments received during public consultation

A comment was received from one MSCA:

They proposed that cyflumetofen should be discussed with regard to classification for reproductive toxicity and that Category 2 for fertility (H361f) should be considered. This was based on their observation that, while no effects on reproductive and fertility index are observed in the 2-gen study, delay in sexual maturation (possibly related to changes in steroidogenesis) is observed in both sexes in the absence of systemic toxicity. Note that there was no effect on bodyweight in all tested groups of any generation, only effects on adrenals are reported in mid and high dose groups which is probably also related to changes in steroidogenesis.

Therefore, a number of significant endpoints were affected in a statistically significant manner.

This was demonstrated by delayed sexual maturation in both male and female offspring (delayed vaginal opening and prepubital separation). It was also observed decreased steroidogenesis (progesterone and 17β-estradiol) in females from 500 ppm, vacuolation of the adrenal cortex apparent in parental animals and offspring, and ovary interstitial vacuolation (1500 ppm), at dose levels not otherwise systemically toxic. They questioned the validity of disregarding statistically significant findings of this nature on the basis that the alterations are within the HCD ranges and therefore not toxicologically relevant.

Assessment and comparison with the classification criteria

RAC Assessment of the reproductive toxicity study

Note

-The units of mg/kg bw/day in food should be replaced with ppm.

-The CLH report in Table 102 gives the NOAEL (development) at 10 mg/kg bw/day based on post-natal adrenal effects (rat 2-generation study). The RAC considers this to be systemic toxicity and 10 mg/kg bw/day to be a systemic NOAEL for offspring. The developmental NOAEL is > 100 mg/kg bw/day.

-Table 103 of the CLH report has points 1 – 5 in the legend which don't seem to apply to this study.

-The introduction table under 4.11.1.1 is inconsistent with Table 102 of the CLH report (Different NOAELs for reproduction). It's also inconsistent with the conclusion (p. 126).

There was no general toxic effect of administration of 0 (control), 150, 500, or 1500 ppm for two successive generations in a well conducted and reported study. There were no changes detected between F0 and F1 parental animals of the treated and control groups in mating indices, pregnancy rates, duration of gestation and male fertility. Likewise, no treatment-related changes were detected in litter size, post-implantation loss, live birth index, viability index, lactation index, sex ratio, clinical signs or pup weight of the F0 offspring (F1 pups) or the F2 pups. In the F2 pups mean body weights were decreased (systemic toxicity from day 7) during lactation at 1500 ppm and anogenital distance was significantly increased (not toxicologically relevant) in males at 150 ($p < 0.01$) and 1500 ($p < 0.05$) ppm. F2 pup mean body weights were not different from controls at birth.

A number of factors were affected by treatment at the 500 ($p < 0.05$) and 1500 ($p < 0.01$) ppm groups which may be considered potentially relevant to reproduction at dose levels causing no other significant toxicity.

1. Significantly increased incidence of white colour of adrenals, increased absolute and relative weights of adrenals and increased diffuse hyperplasia of the zone glomerulosa in F0 and F1 males at 1500 ppm and from 500 ppm in females were noted.

2. The adrenals of F1 and F2 pups from 500 ppm showed significant increases in weights and histopathological findings such as diffuse hypertrophy in zona glomerulosa and/or zona fasciculata. F2 pups in the 1500 ppm group showed white in colour of the adrenals. These findings in adrenals were consistent with observations in parental animals.

3. Examination of ovaries revealed an increased mean weight (F0 and F1) and significantly higher vacuolation of interstitial cells ($p < 0.1$) in F1 parental females of the 1500 ppm group than controls.

4. The mean oestrous cycle length in the F1 1500 ppm group was slightly but significantly ($p < 0.05$) longer than that in the controls.

   Control: 4.0, high dose group (1500 ppm): 4.2 days

   HCD mean: 4.15; HCD range: 4.0 – 4.3 days

5. Sexual development of F1 parental males: increased mean days for completion of preputial separation at 1500 ppm ($p < 0.01$) while body weight was comparable to control.

   Control: 40.9 ± 1.5, high dose group: 41.9 ± 1.5 days

   HCD mean: 41.76; HCD range: 40.7 – 42.5 days

6. Sexual development in F1 parental females: statistically significant increases in the mean days of age at completion of vaginal opening at 500 ppm ($p < 0.05$) and 1500 ppm ($p < 0.01$), while the mean body weight on the completion day in both of the groups were comparable to that in controls.

   Control: 29.5 ± 2.2, medium dose (500 ppm): 30.7 ± 2.1 and high dose (1500 ppm): 31.0 ± 1.9 days

   HCD mean: 30.8; HCD range: 28.2 – 31.8 days

7. Concentrations of FSH in the 500 and 1500 ($p < 0.05$) ppm groups, progesterone in the 150 ($p < 0.05$), 500 ($p < 0.01$) and 1500 ($p < 0.01$) ppm groups, and 17β-estradiol in the 1500 ($p < 0.05$) ppm were significantly lower in F1 dams than the corresponding values in the control group, respectively.

Table: Summary of maternal and offspring effects

Dose ppm	0		150		500		1500	
	M	F	M	F	M	F	M	F
Maternal								
Organ weights	-	-	-	-	-			
-Adrenals	-	-	-	-	-	↑	↑	↑
-Ovaries	-	-	-	-	-	↑	-	↑
-thyroid	-	-	-	-	-	-	-	↑
Histology								
-hypertrophy, zona glomerulosa	3/23	2/23	2/23	3/23	6/21	0/21	**12/24**	**10/23*****
-hypertrophy, zona fasciculate	1/23	4/23	0/23	4/23	8/21	4/21	**22/24**	**16/23*****
-ovary, interstitial cell vacuolation	-		-		-		**-**	
F0		0/24		0/24		0/24		1/24
F1		2/24		2/24		3/24		**14/24*****
Oestrus cycle length (days)	-		-		-		-	
F0	-	4.0	-	4.0	-	4.0	-	4.1
F1		4.0		4.0		4.0		**4.2***
Steroid hormones (F1 parents)								
-FSH (ng/ml)	-	7.6±0.9	-	9.3±1.1	-	**3.7±2.1***	-	**3.7±1.7***
-progesterone (ng/ml)	-	15.7±4.1		**11±4.7***		**10±3.5****		**8.3±2.4****
-17β-estradiol (pg/mL)		19±4.9		18.6±6		17.4±4.8		**12.7±4.9***

<u>Sexual dev. (F1)</u>								
-preputial separation	40.9	-	40.5	-	41.2	-	**41.9***	-
-vaginal opening	-	29.5	-	30.0	-	**30.7***	-	**31.0***
Offspring								
-adrenal weight	-	-	-	-	-	↑	↑	↑
<u>Histology</u>								
-hypertrophy, zona glomerulosa	4/24	1/24	5/24	5/24	**9/24**	7/24	**11/24**	**21/24**
-hypertrophy, zona fasciculata	0/24	6/24	0/24	8/24	0/24	7/24	0/24	**13/24**
-vacuolation, zona fasciculata	3/24	0/24	4/24	0/24	3/24	0/24	**8/24**	0/24
-ovary, interstitial vacuolation	-	2/24	-	2/24	-	3/24	**-**	**14/24**

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.1 ↑ statistically significant in F0 and F1

The clear effects on adrenal weight and adrenal cortex histology in the rat (consistent with the sub-chronic and chronic data) are concurrent with a reduction in the steroid hormones progesterone and 17β-estradiol and could support a mechanism for the observations of delayed sexual development in both male and female F1 animals, i.e., the observed effects can be explained by impaired steroidogenesis. It is acknowledged that the findings are dose-related, statistically significantly different from concurrent controls and most likely treatment related (both in the study and by the RMS). However, it is clear that there is no adverse effect on fertility or reproduction as a consequence and therefore classification is not required as no adverse effect/functional deficit occurred. *RAC notes that the high dose (of 100/140 mg/kg bw/day dietary) may not be high enough as, other than effects on the adrenals, no systemic toxicity was elicited even at the top dose* (whereas the high dose in the developmental toxicity study was 1000 mg/kg bw/day by gavage (York, 2001)). <u>The possibility that more pronounced effects, possibly adverse, could occur at higher doses was not examined</u>. Also, the argument that the various increased values are within the HCD range is less relevant if the effects are considered to be treatment-related. The altered parameters should be compared to the HCD mean not just to its range.

The RMS considers the following as the appropriate adverse levels (LOAELs)

<u>Parental toxicity</u> at 500 ppm based on increased adrenal weight and hypertrophy of adrenal cortical cells. This is in agreement with the conclusion of the DS.

<u>Offspring LOAEL</u> at 500 ppm (35 mg/kg bw/day) based on increased adrenal weights and histopathology in offspring. This was considered a developmental LOAEL by the DS. **RAC does not agree**; pup weights were equivalent to control at birth; these findings are systemic toxicity manifested during lactation and most likely related to ingestion of chow from day 7 onwards. **This is therefore a systemic toxicity LOAEL for offspring.**

<u>Reproductive LOEL</u> > 500 ppm (≈35 mg/kg bw/day) based on no effect on reproduction other than slightly increased oestrus length and reduced steroid hormone levels without adverse effect. Delayed sexual development is considered under the reproduction criteria (CLP Guidance, 2017). Delayed sexual development was seen in F1 males and females at ≥ 500 ppm and was considered treatment-related (but slight and not associated with an adverse outcome).

Although the adrenal (and to some extent the ovaries) are target organs of cyflumetofen, it can be concluded that the effects observed in the 2-generation reproduction study and in the other repeated dose studies, do not lead to *adverse* effects in general, on fertility or its

progeny secondary to the changes in endocrine function.

Developmental toxicity

Rat developmental toxicity

A developmental toxicity study was performed in rats in accordance with OECD TG 414. Female rats were mated and administered 0, 50, 250 or 1000 mg/kg bw/day cyflumetofen by gavage (purity 97.67%) from day 6-19 of gestation. The only maternal toxicity seen was reduced body weight gain (77% of control) and increased adrenal weights and vacuolation of adrenal cortical cells from 250 mg/kg bw/day. Reproduction endpoints were unaffected by treatment and no treatment-related malformations occurred. Skeletal examination showed a delayed ossification at 250 and 1000 mg/kg bw/day as shown by incompletely ossified sternal centra. Foetal incidence of wavy ribs was increased ($p < 0.01$) at 1000 mg/kg bw/day, the litter incidence was not increased however. No other gross external, soft tissue or skeletal variations were increased by treatment. In conclusion, some skeletal variations occurred concurrent with effects on maternal adrenal glands from 250 mg/kg bw/day.

Table: Summary of maternal and offspring effects in the rat developmental study

Dose mg/kg bw/day	0	50	250	1000
Maternal bw change (net)	27.1±8.8	27.2±9.9	26.2±7.1	20.9±8.4*
Adrenal wt (abs) -Right -Left	0.045±0.008 0.048±0.008	0.047±0.009 0.051±0.009	**0.049±0.009*** 0.055±0.008	**0.06±0.010**** **0.064±0.011****
Adrenals (rel) -Right -Left	13.0±2.2 13.9±2.0	13.9±2.0 14.7±2.7	13.9±2.3 **15.5±2.7***	**17.9±2.9**** **18.6±2.9****
Histology -hypertrophy, cortical cell, diffuse, bilateral -vacuolation, cortical cell, diffuse, bilateral	0 0	0 0	0 2	**14** **24**
Foetal Sternal centra: incompletely ossified Litter N (%) Foetal N (%)	4 (16.7) 5 (3.3)	2 (8.7) 4 (2.7)	**9 (39)**** 13 (7.9)	**9 (36)*** 13 (8.3)
HCD: 17 studies June 1998-June 2000, 406 litters, 3051 foetuses Litter; 50 (12.32%), 1 – 5 (4.2% - 21.7%). Foetal; 61 (2%), 1 – 7 (0.6% – 4.0%)				
Ribs: Wavy Litter N (%) Foetal N (%)	5 (21) 6 (3.9)	3 (13) 5 (3.4)	4 (17) 9 (5.5)	7 (28) **16 (10)****
HCD: Litter; 5 (1.23%), 0-1 (0 – 4.8%). Foetal; 5 (0.16), 0-1 (0 – 0.6%)				

*p≤0.05; ** p≤0.01

Rabbit developmental toxicity

A developmental toxicity study was performed in rabbits in accordance with OECD TG 414. Female rabbits were mated and administered 0, 50, 250 or 1000 mg/kg bw/day cyflumetofen by gavage (purity 97.67%) during days 6-28 of gestation. Absolute liver weights were decreased from 250 mg/kg bw/day without statistical significance (7.8% and 8.3%, respectively) and adrenal weights were reduced at 1000 mg/kg bw/day (right 11.9% and left 6.3%). Body weight gains were reduced for the entire gestation period from 250 mg/kg bw/day (24.3% and 32.4%, respectively), also without statistical significance, concurrent with

reduced food consumption.

A number of foetal parameters were altered at 1000 mg/kg bw/day. Reduced foetal body weights were associated with significantly increased incompletely ossified 1st sternal centre and significantly increased litter incidences of angulated hyoid alae. All are commonly occurring variations which can be increased in incidence with reduced foetal weights.

Table: Summary of maternal and offspring effects in the rabbit developmental study

Dose (mg/kg bw/day)	0	50	250	1000
Maternal weight gain				
6-9	+0.01	+0.00	-0.02	-0.03
6-29	+0.37	+0.34	**+0.28 (-24.3%)**	**+0.25 (-32.4%)**
6-29 (Corrected for gravid uterus)	-0.17	-0.17	**-0.23 (-35%)**	**-0.26 (-53%)**
Foetal weight (g/litter)	46.96 ±5	45.56±5.5	45.4±6.2	**41.1±4.4****
Skeletal findings *Litter incidence:* - incompletely ossified sternal centra	0	0	1 (4.5%)	**8 (32%)****
Foetal incidence: - incompletely ossified sternal centra	0	0	1 (0.6%)	**16 (7.3%)****
HCD: 67 studies Jan 1999-2001, 885 pregnant dams at sectioning *Litters; mean 14 (1.89%), range 0-2 (0-10.5%) in 11/44 studies. Foetal; mean: 15 (0.24), range 0-2 (0-1.2%)*				
Litter incidence: - hyoid, angulated alae	2 (8.3%)	2 (8.3%)	2 (8.3%)	**10 (40%)****
Foetal incidence - hyoid, angulated alae	3 (1.6%)	3 (1.5%)	3 (1.7%)	**11 (5%)**
In house HCD (Jan 1999-2001) *Litters; mean 94 (12.70%), range 0-6 (0-31.6%) in 36/44 studies. Foetus; mean 111 (1.76%), range 0-10 (0-6.4%)*				

Comparison with the criteria

Sexual function and fertility

Arguments for classification

There were a number of treatment-related effects on reproduction associated parameters which are relevant to classification, as described above. All effects were statistically significant and a treatment and dose-relationship was apparent. A plausible mechanism based on a targeted effect on lipid metabolism and steroidogenesis is supported by the pattern of effects. Comparison to the HCD shows that many endpoints are slightly above the mean value but within the range of values recorded. It is noted that where the effects are believed to be treatment related, a comparison to HCD is less relevant. It is further noted that the maximum dose in the rat 2-generation study was limited to about 140 mg/kg bw/day. More pronounced effects may have been observed if the doses were greater than those tested.

Argument against classification

Even though a plausible mechanism could be supported, the effects were relatively slight at the maximum dose tested. Most importantly, there was no functional deficit on fertility or reproduction in either generation. There is no clear evidence of an adverse effect on sexual function and **Cat. 1B is not supported**.

The study was well conducted and no deficiencies were detected, however the choice of dose levels may be questioned. In this study, rats were only dosed up to a maximum of 140 mg/kg bw/day. It is not clear why higher doses were not tested, especially since much higher doses of 250 – 1000 mg/kg bw/day were tolerated by rodents and rabbits in the developmental studies. There is thus a lack of sufficient evidence to support classification. As the effects seen do not impact on fertility or reproductive function, **Cat. 2 is not supported**.

Developmental toxicity

Arguments for classification/non-classification

In the rat study, some delayed ossification and increased incidence of wavy ribs occurred concurrent with significantly reduced maternal weight gain and effects on maternal adrenal glands from 250 mg/kg bw/day. There were no increases in malformations or other adverse developmental findings. **Classification in Cat. 1B is not supported by this study**.

In the rabbit study, some effects on maternal weight gain and adrenal weight were seen from 250 mg/kg bw/day (probably treatment-related but not statistically significant). At the high dose level, foetal weight was significantly reduced ($\approx$10%) in conjunction with reduced ossification and an increase in hyoid alae change with no increase in malformations. Reduced foetal weight in one of two species tested is **not sufficient evidence of adversity to classify in Cat. 1B**.

In both species there was an increase in commonly occurring skeletal variations at the high dose causing some maternal toxicity. In addition, foetal weight was decreased in the rabbit. These findings were considered for Cat. 2. However, it must be noted that there were no increases in malformations in either of the two species tested. In conclusion, an increase in commonly occurring skeletal variations were observed. Although treatment-related, these effects are not considered by themselves sufficient to meet the criteria for adversity, they are not considered permanent developmental effects and **classification in Cat. 2 is not supported**.

In conclusion, RAC agrees with the DS that **classification is not warranted for sexual function and fertility or for development of the offspring**.

4.12   Other effects

4.12.1   Non-human information

4.12.1.1   Neurotoxicity

There have been no indications of neurotoxicity observed in the acute or repeated dose studies which have been performed with cyflumetofen and therefore neurotoxicity studies are not considered necessary. However, an acute neurotoxicity study and 90-day study are available and shortly summarized.

Table 117 Summary table

Method	Results	Remarks	Reference
Acute neurotoxicity study in rat, Wistar (oral gavage) 0 or 2000 mg/kg bw/d	Cyflumetofen does not affect general condition nor behaviour after a single dose of 2000 mg/kg bw	-	Buesen, 2010
Subchronic neurotoxicity study in rat, Wistar 0, 500, 1500 or 5000 mg/kg food, equal to 0, 30, 89 and 293 mg/kg bw/d for males and 0, 41, 99 and 353 mg/kg bw/day for females	No neurobehavioral effects observed. NOAEL: 293 mg/kg bw/d (males) and 353 mg/kg bw/d (females).	-	Buesen, 2011

Acute neurotoxicity study in rats (Buesen, 2010)

Six male Wistar rats were given a single dose of 0 or 2000 mg/kg bw cyflumetofen (purity 97.67%) by gavage. At 2000 mg/kg bw, only urination and defecation were noted, however, these observations were also made in the control group. No further changes in general condition and behaviour were noted. It is concluded that cyflumetofen does not affect general condition nor behaviour after a single dose of 2000 mg/kg bw. Overall it can be concluded that cyflumetofen does not have neurotoxic potential.

Subchronic neurotoxicity study in rats (Buesen, 2011)

The oral administration of cyflumetofen to Wistar rats at dose levels of 0, 500, 1500 or 5000 ppm over a period of 3 months revealed no adverse neurobehavioral effects in male and female Wistar rats at any concentration. In addition, no test substance-related effects were observed in the neurohistopathology investigation at any concentration. Under the conditions of this study the no observed adverse effect level (NOAEL) for neurotoxicity was 5000 ppm for male (293 mg/kg bw/d) and female animals (353 mg/kg bw/d).

The NOAEL for general systemic toxicity was 500 ppm for male (41 mg/kg bw/d) and female animals (30 mg/kg bw/d) based upon pathological changes in adrenal glands.

4.12.1.2 Immunotoxicity

No indication for immunotoxic effects was obtained from any of the repeated dose studies. Nevertheless, an immunotoxicity study was performed and is shortly summarized below.

Table 118 Summary table

Method	Results	Remarks	Reference
28-day immunotoxicity study in rat, Wistar (females) 0, 4.5, 33, 107 or 349 mg/kg bw/d	No immunotoxicological effects seen. NOAEL: 349 mg/kg bw/d	-	Buesen, 2010

28 day immunotoxicity study in rats (Buesen, 2010)

Cyflumetofen was administered to groups of 8 female Wistar rats at dose levels of 0 ppm, 500 ppm, 1500 ppm and 5000 ppm via the diet over a period of 4 weeks. All animals were immunized 6 days before blood sampling and necropsy using 0.5 mL sheep red blood cells (4×108 SRBC/mL), administered intraperitoneally. Under the conditions of the study Cyflumetofen did not reveal any signs of immunotoxicity. The NOAEL for the immunotoxicologically relevant endpoints was set to 5000 ppm (349 mg/kg bw/d) - the highest dose tested. The NOAEL for systemic toxicity was set to 500 ppm (33 mg/kg bw/d) based on treatment-related changes in the adrenal glands at 1500 ppm (107 mg/kg bw/d) and higher.

4.12.1.3 Specific investigations: other studies

Mechanistic data

To elucidate the mechanism(s) for the effects on adrenal gland and ovary, a mechanistic study was performed (Takeda, 2006). Furthermore, a study in dogs was performed (Inui, 2003) to study possible pharmacological effects of cyflumetofen. This provides some additional information, but is not considered relevant for the overall risk assessment.

Table 119 Summary table

Method	Results	Remarks	Reference
28-day oral toxicity study in rat, Fischer (F344/DuCrlCrj) (mechanistic) 0, 100 or 5000 mg/kg food, equal to 0, 7.44 and 378 mg/kg bw/d for males and 0, 7.59 and 347 mg/kg bw/d for females	Vacuolation of adrenal cortical cells and vacuolation of interstitial ovary cells observed after repeated exposure to cyflumetofen is probably due to cholesterol deposition as a result of a reduction in hormone-sensitive lipase	-	[a]Takeda, M., 2006
24 hours pharmacology test in dog, Beagle 0 or 2000 mg/kg bw	Cyflumetofen does not induce effects on the respiratory and cardiovascular system in dogs at 2000 mg/kg bw.	-	[a]Inui, T., 2003b

28-day oral mechanistic study in rats (Takeda, 2006)

Reference	:	Takeda, M., 2006	exposure	: 28 days, diet
type of study	:	28-day oral toxicity study	dose	: 0, 100 or 5000 mg/kg[1] food
year of execution	:	2006	vehicle	: None
Test substance	:	OK-5101 (batch 01D1; purity 97.7%)	GLP statement	: No
Route	:	Oral	guideline	: No guideline applicable
Species	:	Rat, Fischer (F344/DuCrlCrj)	acceptability	: Acceptable
group size	:	10/sex/dose (control and 5000 mg/kg food group); 8/sex (100 mg/kg food group)		

[1] equal to 0, 7.44 and 378 mg/kg bw/d for males and 0, 7.59 and 347 mg/kg bw/d for females

To elucidate the mechanism(s) for the effects on adrenal gland and ovary, a mechanistic study was performed. Rats were given cyflumetofen for 4 weeks at dose levels of 0, 100 or 5000 mg/kg food (equal to 0, 7.44 and 378 mg/kg bw/d for males and 0, 7.59 and 347 mg/kg bw/d for females). The control group and 5000 mg/kg food group consisted of 10 animals per sex; the 100 mg/kg food group consisted of 8 animals per sex. Dose levels were based on the 28-day study in rats, in which a significant increase in adrenal gland weight for both sexes, an increased ovary weight in females and histopathological changes in the adrenals in both sexes were noted at 5000 mg/kg food.

Observations for mortality and morbidity were made twice daily and clinical examinations were made once per day. Body weights were recovered for all animals before treatment and once weekly. Food consumption was measured before initiation of treatment and weekly during the treatment period. Serum hormone levels were measured including adrenocorticotropic hormone (ACTH) and corticosterone after 4 weeks. After 4 weeks of treatment, all surviving animals were subjected to necropsy. All gross findings were recorded. Adrenal and ovary weights were measured. Two animals of each sex of the 0 and 5000 mg/kg food groups were subjected to ultra-structural examination on the adrenal gland and/or ovary. From the remaining animals (8 animals/sex/group), bilateral adrenal glands and ovaries were subjected to histopathological examination. Frozen samples of the adrenal glands (half of the left adrenal) were used for RNA extraction, confirmation of integrity and purity of extracted RNA and quantitative analyses of gene expression. Quantitative analyses were performed on the following genes: CYP11A1, CYP11B1, NCEH (neutral cholesteryl ester hydrolase) and HSL (hormone-sensitive lipase). GAPDH (glyceraldehydes-3-phosphate dehydrogenase was used as internal standard to normalize gene expression levels. Measurement of cholesterol in the adrenal gland was performed on all animals using a sample of the right adrenal gland. In Table 120 the results are summarized.

Table 121 Results

Dose (mg/kg food)	0		100		5000		dr
	m	f	m	f	m	f	
Mortality	None						
Clinical signs	No treatment-related findings						
Body weight	No treatment-related findings						
Food consumption	No treatment-related findings						
Serum hormone levels	No treatment-related findings						
Gene expression adrenal (ratio to control values)							
- GAPDH			101	99	101	99	
- CYP11A1			98	110	124	133**	
- CYP11B1			90	99	114	70**	
- NCEH			181	71	125	100	
- HSL			87	81	79	69**	
Gene expression adrenal (ratio to GAPDH)							
- CYP11A1			98	111	125*	136**	
- CYP11B1			90	101	113	70*	
- NCEH (*1/100)			169	72	123	98	
- HSL (*1/10)			86	82	78*	69**	
Cholesterol in adrenal gland (% of control)							
- total cholesterol			84	98	124*	154**	
- free cholesterol			86	102	120	139**	
- cholesteryl ester			76	78	145	226	
Organ weights							
- adrenals					ic[a,r]	ic[a,r]	
- ovary						ic[a], i[r]	

Dose (mg/kg food)	0		100		5000		dr
	m	f	m	f	m	f	
Pathology							
macroscopy							
- adrenal, enlargement	0/10	0/10	0/8	0/8	10/10	10/10	
- adrenal, white in colour	0/10	0/10	0/8	0/8	10/10	10/10	
microscopy							
adrenal:							
- vacuolation, cortical cell, diffuse							
ovary:	0/8	0/8	0/8	0/8	8/8	8/8	
- vacuolation, interstitial cell		0/8		0/8		8/8	

dr   dose related
dc/ic   statistically significantly decreased/increased compared to the controls
d/i   decreased/increased, but not statistically significantly compared to the controls
a/r   absolute/relative
* $p \leq 0.05$
** $p \leq 0.01$

No mortality, no treatment-related findings on clinical signs, body weight and food consumption were noted. There were no significant changes in serum ACTH and corticosterone levels.

All animals in the 5000 mg/kg food group had enlarged and whitish adrenal glands. No changes were noted at 100 mg/kg food. Absolute adrenal weights were significantly increased in males and females at 5000 mg/kg food (126 and 165% of controls, respectively). Relative adrenal weights were also significantly increased in males and females at this dose level (120 and 164% of controls). Both absolute and relative ovary weights were slightly increased at 5000 mg/kg food (111 and 110% of controls). Since in two animals at 5000 mg/kg food large cysts were observed in the ovaries, the ovary weight was re-evaluated, excluding the weights of the ovaries with cysts, resulting in an even smaller difference in ovary weights between control and high dose females (107% of control for absolute weight; 105% of controls for relative weight).

Quantitative analysis of gene expression in the adrenal gland revealed for the absolute gene expression levels a significant increase in CYP11A1 and significant decreases in CYP11B1 and HSL in females at 5000 mg/kg food. As to the ratio to GAPDH of gene expression in the adrenal gland, a significant increase in CYP11A1 and a significant decrease in HSL were observed at 5000 mg/kg food in males and females. In addition, females at 5000 mg/kg food showed a significant decrease in CYP11B1. These changes were almost the same as those in the absolute values of gene expression. Therefore, the increase in CYP11A1 and the decreases in HSL and CYP11B1 in males and/or females at 5000 mg/kg food are considered to be toxicological significant.

At 5000 mg/kg food, males and females showed an increase in total cholesterol, free cholesterol and cholesteryl levels in the adrenal gland.

Histopathologically, diffuse cytoplasmic vacuolation of cortical cells in the adrenal gland was observed in males and females at 5000 mg/kg food. In addition, vacuolation of interstitial cells in the ovary was observed in all females of 5000 mg/kg food.

Ultra structural observation revealed an increase in the number of cytoplasmatic lipid droplets of adrenal cortical cells at 5000 mg/kg food in both sexes, and the size of the lipid droplets observed in males was larger than in females. An increase in the number of cytoplasmatic lipid droplets was also noted in the ovarian interstitial cells at 5000 mg/kg food.

Dietary administration of cyflumetofen at 5000 mg/kg food resulted in an increase in adrenal weight and vacuolation of adrenal cortical cells and ovarian interstitial cells. Ultra structural observation revealed that the vacuolation was associated with the deposition of lipid droplets in the cytoplasm.

In addition, electron microscopic evaluation showed that the adrenal cortical cell vacuolation observed in males is fundamentally the same effect described as adrenal cortical cell hypertrophy in females, the only difference being the size of lipid vacuoles. These morphological alterations were consistent with the increased cholesterol levels in the adrenal gland. Quantitative analysis of gene expression in the adrenal gland revealed a significant decrease in hormone-sensitive lipase (HSL) at 5000 mg/kg food of both sexes. Since HSL is a major enzyme involved in cholesterol metabolism and regulates cholesterol hydrolysis in the adrenal gland, the decrease in HSL might result in inhibition of hydrolysis, leading to cholesterol deposition in adrenals, which would be consistent with the lipid deposition observed. A similar mechanism is probably present in ovaries. The increase in CYP11A1 in both sexes at 5000 mg/kg food is considered a secondary response to dispose of cholesterol deposited in the adrenal gland since CYP11A1 is a cholesterol side chain cleavage enzyme converting cholesterol to pregnenolon. The decrease in CYP11B1 observed in females at 5000 mg/kg food was considered of no toxicological significance since there was neither a change in males nor a change in serum ACTH (adrenocorticotropic hormone) or corticosterone for either sex. Hormone-mediated secondary effects on the adrenal and ovary are not likely to occur, because there were no abnormalities in serum ACTH and corticosterone levels and no increase in CYP11B1 for either sex. The threshold for the described mechanism lies between 100 and 5000 mg/kg food (i.e. between 7.44 and 378 mg/kg bw/d for males and 7.59 and 347 for females). In conclusion, the vacuolation of adrenal cortical cells and vacuolation of interstitial ovary cells after repeated exposure to cyflumetofen is probably due to cholesterol deposition as a result of a reduction in hormone-sensitive lipase.

Pharmacology study (Inui, 2003)

Reference	:	Inui, T., 2003b	exposure	:	24 hours or 1 week, gelatine capsule
type of study	:	24 hours pharmacology test	dose	:	0, 2000 mg/kg bw
year of execution	:	2003	vehicle	:	None
test substance	:	OK-5101 (batch 01D1; purity 97.7%)	GLP statement	:	Yes
Route	:	Oral	guideline	:	none
Species	:	Dog, beagle	Acceptability	:	supplementary
group size	:	4 males			

The possible pharmacological effects of cyflumetofen were studied by measuring respiratory rate, blood pressure, heart rate, and electrocardiogram in conscious dogs. Male dogs were given a single oral dose of 0 or 2000 mg/kg bw. In total 4 animals were used. Animals received both the control and the 2000 mg/kg bw capsules with a withdrawal period of 6 days after each administration. The respiratory rate, blood pressure, heart rate, and electrocardiogram parameters (PQ interval, QRS duration, QT interval, and QTC) were determined before and 0.5, 1, 3, 6 and 24 hours after administration.

Systolic blood pressure, heart rate, the electrocardiogram (PQ interval, QRS duration, QT interval, QTc) and the respiratory rate in the 2000 mg/kg bw group showed no statistically significant difference when compared to controls at 0.5, 1, 3, 6 and 24 hours after administration. Diastolic blood pressure and mean blood pressure at 1 hours after administration were higher in the cyflumetofen treated group when compared to controls, however, values remained within the range of post-administration measurements in the vehicle control group and considered unrelated to treatment with cyflumetofen. It is concluded that cyflumetofen does not induce effects on the respiratory and cardiovascular system in dogs at 2000 mg/kg bw.

Tier 1 tests of the Endocrine Disrupting Screening Program

Cyflumetofen was tested in a battery of screening tests for endocrine disrupting potential, according to the TIER 1 tests of the Endocrine Disrupting Screening Program (EDSP) of the US EPA.

Table 122 Summary table

Method	Results	Remarks	Reference
Aromatase (Human Recombinant) Assay (US EPA OPPTS 890.1200)	The maximum extent of CYP19 aromatase activity inhibition was in range of 4% to 9%.	The Aromatase assay is in the Tier 1 screening battery of the Endocrine Disruptor Screening Program (EDSP) of the US EPA. Cyflumetofen is not an inhibitor of CYP19 aromatase activity.	[a]Foster J. 2011

Method	Results	Remarks	Reference
Oestrogen Receptor Transcriptional Activation (Human Cell Line (HeLa-9903)) US EPA OPPTS 890.1300	Negative overall ERα activation response.	No evidence of ERα transcriptional activation in this test system.	[a]Akhurst, L 2011
H295R Steroidogenesis Assay US EPA OPPTS 890.1550	Estradiol induction: NOEC 1 µM; LOEC 5 µM Testosterone inhibition: NOEC 100 nM; LOEC 1 nM	This assay is part of the Tier 1 screening battery of the Endocrine Disruptor Screening Program (EDSP) of the US EPA. Cyflumetofen acts as inducer of estradiol production and inhibitor of testosterone production in this system.	[a]Akhurst, L 2011
Estrogen Receptor Binding Assay Using Rat Uterine Cytosol (ER-RUC) US EPA OPPTS 890.1250	Negative in the tested range of $1 \times 10^{-10} – 1 \times 10^{-4}$ M	This assay is part of the Tier 1 screening battery of the Endocrine Disruptor Screening Program (EDSP) of the US EPA Cyflumetofen is classified as 'not interactive' for ER binding	[a]Akhurst, L 2011
Androgen Receptor Binding (Rat Prostate Cytosol) US EPA OPPTS 890.1150	Cyflumetofen's mean specific binding was > 98%, >85% and > 83% respectively for the three independent runs, classifying Cyflumetofen as a "non-binder".	This assay is part of a Tier 1 battery which is intended for screening purposes only and should not be used for endocrine classification or risk assessment. Cyflumetofen is considered a "non-binder" for the Androgen receptor.	[a]Willoughby,J 2012

[a]As summarised by OECD 2012 section 3 Annex II Document M

Aromatase (Human Recombinant) Assay (Foster, 2011)

Cyflumetofen was assayed for its ability to inhibit aromatase (CYP19) activity, using the EPA recommended methodology. Cyflumetofen (final concentration: 10-4.5 to 10-10 M) was compared with a positive control, 4-hydroxyandrostenedione (formestane), to determine the extent of inhibition of aromatase activity. The maximum extent of inhibition of CYP19 aromatase activity by

cyflumetofen was between 4% and 9% (91% to 96% of full activity control), in three runs. Based on these findings cyflumetofen is not an inhibitor of CYP19 aromatase activity.

Estrogen Receptor Transcriptional Activation (Akhurst, 2011)

To determine the potential for cyflumetofen to function as an estrogen receptor α (ER α) ligand by activating an agonist response in a human cell line (HeLa- 9903), ERα activation was determined using a luciferase activity assay system. The highest (non-cytotoxic and soluble) concentration of cyflumetofen used was 10 µM. Cyflumetofen did not show evidence of ERα transcriptional activation in this test system.

Steroidogenesis Assay (Akhurst, 2011)

The steroidogenesis assay examines the effects of graded concentrations of the test material to affect the conversion of cholesterol to estradiol and or testosterone in human H295R cells. Cyflumetofen demonstrated an apparent influence on the steroidogenesis pathway, acting as an inducer of estradiol production and an inhibitor of testosterone production in this test system. The LOEC for the overall study for estradiol induction was 5 µM, with a NOEC of 1 µM. The LOEC for the overall study for testosterone inhibition was 1 µM, with a NOEC of 100 nM. However, the biological significance of the low levels of change in steroid metabolism is equivocal, and should not be considered to indicate potential for endocrine disruption in the absence of significant similar changes or adverse consequences in vivo.

Oestrogen Receptor Binding Assay (Akhurst, 2011)

The potential for Cyflumetofen to interact with oestrogen (estrogen) receptors isolated from rat uteri, through measurement of its displacement of radiolabelled ligand ([3H]17β-oestradiol) was determined, using the EPA recommended methodology. The concentrations of cyflumetofen tested were in the range of $1 \times 10\text{-}10$ to $1 \times 10\text{-}4$ M. Based on the results of three assay runs, Cyflumetofen was classified as 'not interactive' for ER binding.

Androgen Receptor Binding (Willoughby, 2012)

To detect potential interaction with the androgen hormonal system, cyflumetofen was tested in the Androgen Receptor Binding (Rat Prostate Cytosol). The suitable top concentration of cyflumetofen for use in the binding assays was 10-4 M. Cyflumetofen's mean specific binding was > 98%, >85% and > 83% respectively for the three independent runs, classifying cyflumetofen as a "non-binder" for the Androgen receptor.

4.12.2 Summary and discussion

In addition to absence of indications of neurotoxicity in the acute or repeated dose studies with cyflumetofen, also an acute neurotoxicity study with male rats given a single dose of 0 or 2000 mg/kg bw by gavage as well as a subchronic neurotoxicity study in male and female Wistar rats at dose levels of 0, 500, 1500 or 5000 ppm showed no neurotoxic potential.

Cyflumetofen did not induce any signs of immunotoxicity when administered via the diet over a period of 4 weeks to female Wistar rats. The NOAEL for the immunotoxicologically relevant endpoints was set to 5000 ppm (349 mg/kg bw/d) - the highest dose tested. The NOAEL for systemic toxicity was set to 500 ppm (33 mg/kg bw/d) based on treatment-related changes in the adrenal glands at 1500 ppm (107 mg/kg bw/d) and higher.

A mechanistic study was performed to elucidate the nature of the adrenal effects. Rats administered 5000 ppm cyflumetofen for 4 weeks showed increased adrenal weights accompanied by vacuolation of adrenal cells and increased cholesterol content of the adrenal, and vacuolation of ovarian interstitial cells. The observed vacuolation of adrenal cortical cells and vacuolation of interstitial ovary cells after repeated exposure to cyflumetofen is probably due to cholesterol deposition as a result of a reduction in hormone-sensitive lipase. As no abnormalities were observed in serum ACTH and corticosterone levels and no increase in CYP11B1 for either sex, hormone-mediated secondary effects on the adrenal and ovary are not likely to occur. The threshold for the described mechanism lies between 100 and 5000 mg/kg food (i.e. between 7.44 and 378 mg/kg bw/d for males and 7.59 and 347 for females).

Cyflumetofen was tested in a battery of screening tests for endocrine disrupting potential, according to the TIER 1 tests of the Endocrine Disrupting Screening Program (EDSP) of the US EPA. In these tests it was demonstrated that cyflumetofen only acts as an inducer of estradiol production and inhibitor of testosterone production in an *in vitro* system, whereas cyflumetofen is not an inhibitor of CYP19 aromatase activity, shows no estrogen receptor (ERα) transcriptional activation, is not interactive for Estrogen Receptor (ER) binding and is considered a non-binder for the Androgen receptor. However, it should be noted that cyflumetofen is extensively metabolised and that no comparable in vitro tests are available for these metabolites. It is also known that a major metabolite is responsible for the acaricide activity. Therefore, these results do not exclude endocrine disruptive effects in vivo.

4.12.3 Comparison with criteria

There are no criteria for neurotoxicity or immunotoxicity. There effects are covered by the STOT RE classification.

4.12.4 Conclusions on classification and labelling

Classification and labelling is not required.

RAC evaluation of aspiration toxicity

Additional key elements

Cyflumetofen is a yellow, odourless solid of low volatility. The vapour pressure of purified cyflumetofen is $< 5.9 \times 10^{-6}$ Pa at 25 °C, which, combined with a low and pH independent water solubility of 28 µg/L at pH 7, results in a Henry's law constant of $< 9.4 \times 10^{-2}$ Pa.m^3.mol^{-1}. The surface tension was not determined. The substance is not considered to be surface active.

Assessment and comparison with the classification criteria

Based on the properties of the substance, it is not expected to pose an aspiration hazard.

---

5   ENVIRONMENTAL HAZARD ASSESSMENT

The environmental fate properties and hazard assessment for cyflumetofen are based on the European Food Safety Authority (EFSA) conclusion on pesticide peer review ("Conclusion on the peer review of the pesticide risk assessment of the active substance cyflumetofen", EFSA Journal 2012;10(1):2504), being the primary source of information. More detailed data were taken from the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B8 and B9; September 2011.

5.1 Degradation

Table 123 Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis (JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993))	DT_{50} pH 4 = 7.7 days (25°C) DT_{50} pH 5 = 6.0 days (25°C) DT_{50} pH 7 = 9.8 hours (25°C) DT_{50} pH 9 = 10.3 min (25°C)	Metabolites > 10 %: A-1, A-2, A-18, B-1, AB-1	Nakamura, 2004g (IIA7.5/01) [a]
Photolysis, natural and sterilized water (JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993))	DT_{50} natural water: 1.07 hr (25°C) DT_{50} at pH5: 1.28 hr (25°C) Quantum yield 3.0×10^{-5} mole/Einstein	Specific photolytic degradation products > 10 %: AB-7 and AB-15 Aquatic photolysis products (buffer pH 5) > 10%: B-1, AB-7 and AB-15	Ohyama, 2004d (IIA 7.6/01) [a]
Water/sediment system, aerobic (OECD 308)	Cyflumetofen: DT_{50} water = 0.1 days (SW^b system) and 0.7 days (GV^c system) DT_{50} sediment = 2.1 days (SW^b system) and 12 days (GV^c system) DT_{50} system = 0.2 days (SW^b system) and 9.9 days (GV^c system)Metabolite A-2: DT_{50} system: 11.74 days (GV^c system) Metabolite AB-1: DT_{50} system: 18.29 days (GV^c system) Metabolite Met8 DT_{50} system: 3.09 days (SW^b system)	A-ring ^{14}C labelled cyflumetofen; results of 2 water/sediment systems. Relevant (> 10%) metabolites: Met1, A-2, Met8, AB-11.	Noorloos and Brands, 2008 (IIA 7.8.3-01) [a]
Water/sediment system, aerobic (OECD 308)	Cyflumetofen: DT_{50} water = 0.05 days (SW^b system) and 0.1 days (GV^c system) DT_{50} sediment = 0.6 days (SW^b system) and 15 days (GV^c system) DT_{50} system = 0.08 days (SW^b system) and 14 days (GV^c system) Metabolite B-1: DT_{50} system = 320 days (GV^c system) Metabolite B-2: DT_{50} system = 40 days (SW^b system) and 0.77 days (GV^c system)	B-ring ^{14}C labelled cyflumetofen; results of 2 water/sediment systems. Relevant (> 10%) metabolites: B-1, B-2.	Noorloos and de Mol, 2007 (IIA 7.8.3-02) [a]
Photochemical oxidative degradation in air (AOPWIN v 1.91 software; part of US-EPA''s EPI suite vs 3.12 of 2000)	DT_{50} air = 8.2 hours	Based on a 12-h OH-radical concentration of 1.5×10^6 molecules cm^{-3}.	Willems 2008a (IIA 2.10/01) [a]
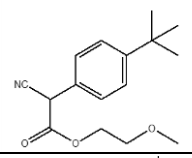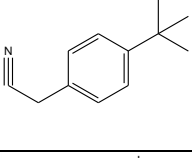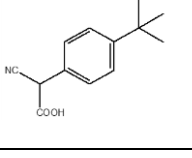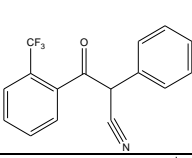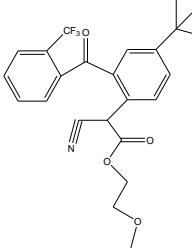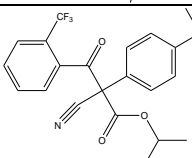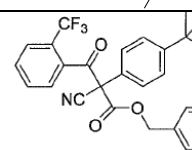
[a] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B.8; September 2011.

[b] Schoonrewoerdsewiel (SW) water/sediment system.

[c] Goorven (GV) water/sediment system.

An overview of the relevant degradation/metabolic products is provided in the table below. Relevant was considered to represent products occurring at levels >10% of AR, or >5% at two consecutive samplings, or increasing until the end of the study. Physical-chemical properties were not available for all products, and these were therefore estimated using established QSARs.

Table 124 Overview of relevant degradation products

Structure	Name	Matrix and maximum formation (% of AR) [a]	Mw	LogPow (KOWWIN v1.68)	Water solubility (mg/L, 25 °C) (Wat Sol v1.01) [c,d]	Vapour pressure (Pa, 25 °C) (MPBPVP v1.43) [c,d]
	A-1	Water: 14.44% (H)	275.4	2.79	286.71 (191.5, ECOSAR v0.99h)	0.000743
	A-2	Water: 44.12% (H) 18.4% (B)	173.3	3.47	62.047 (24.39, ECOSAR v0.99h)	0.374
	A-18	Water: 36.22% (H)	217.26	2.78	1203.1 (154.7, ECOSAR v0.99h)	0.000648
	AB-1	Water: 44.51% (H) Sediment: 14.6%	345.4	5.33	0.14196	4.35E-05 (4.35E-2, MPBPWIN v1.41)
	AB-7	Water: 10.82% (P)	447.5	4.91	0.38077 (2.141 ECOSAR v0.99h)	7.28E-08
	AB-11	Water: 10.0% (B) Sediment: 10.1%	431.5	5.87	0.040 (0.2165, ECOSAR v0.99h)	4.59E-07
	AB-12	Water: 7.6% (B) [b]	535.6	8.58 [c]	2.33E-05	2.48E-010

Structure	Name	Matrix and maximum formation (% of AR) [a]	Mw	LogPow (KOWWIN v1.68)	Water solubility (mg/L, 25 °C) (Wat Sol v1.01) [c,d]	Vapour pressure (Pa, 25 °C) (MPBPVP v1.43) [c,d]
	AB-15	Water: 54.67% (P)	447.5	5.05	0.402 (1.541, ECOSAR v0.99h)	4.16E-09
	B-1	Water: 53.17% (H) 11.88% (P) 65% (B) Sediment: 21.5%	190.1	2.49	196.03 (267.4, ECOSAR v0.99h)	0.3986
	B-2	Water: 15.4% (B) Sediment: 28%	362.23	4.26	0.20481 (7.976, ECOSAR v0.99h)	0.0371
	Met 1 (tentative structure)	Water: 10.7% (B)	392.4	6.21	0.013 (0.08862, ECOSAR v0.99h)	3.6E-05
Unidentified	Met 4	Water: 7.6% (B) [b] Sediment: 10.7%	-	-	-	-
Unidentified	Met 8	Water: 19.5% (B)	-	-	-	-
Unidentified	Met 14	Water: 6.6% (B) [b]	-	-	-	-

[a] Between brackets: H refers to hydrolysis, P refers to photolysis and B refers to aerobic degradation in water/sediment systems

[b] Detected at levels >5% of AR at two consecutive samplings

[c] New calculations, added in response to questions from ECHA, 2016

[d] Between brackets: data included in the revised DAR of September and October 2011, in case of differences between existing and newly generated data

5.1.1   Stability

Hydrolysis

reference	:	Nakamura H. (2004g)	purity	:	Chemical purity not reported, radiochemical purity 99.2 (A-ring), 98.1% (B-ring).
study type	:	Hydrolysis	nominal concentration	:	0.08 mg/L
year of execution	:	2003-2004	pH	:	4, 5, 7, 9
GLP statement	:	yes	temperature	:	25°C
guideline	:	JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993)	Conclusion	:	Susceptible to hydrolysis, DT50 10.3 minutes to 7.7 days. Metabolites > 10 %: A-1, A-2, A-18, B-1, AB-1
test substance	:	[A-ring-U-^{14}C]Cyflumetofen (batch CFQ12735)	acceptability	:	acceptable

[B-ring-U-14C]Cyflumetofen (batch CFQ12736)

Portions of 2.5 mL [A-ring-U-14C]Cyflumetofen or [B-ring-U-14C]Cyflumetofen in acetonitrile were spiked to 250 mL sterilised, deoxygenated buffer solutions of pH 4 (0.1M NaOH/potassium dihydrogen citrate), 5 (0.2M Acetic acid/sodium acetate), 7 (0.1M NaOH/potassium dihydrogen phosphate) and 9 (0.1M NaOH/KCl/boric acid) at a concentration of 0.1 mg/L. Radiopurity and concentration of the spike solutions were determined by HPLC and LSC, respectively. The fortified buffer solutions were incubated in the dark at 25 ± 1°C for 30 days (pH 4, 5 and 7), 10 days (pH 7 B-ring) or 1 day (pH 9). Sterility and pH were checked at the beginning and end of the incubation period. Samples were taken at minimum 7 different time points. Immediately after sampling, the pH 7 and pH 9 samples were acidified (acetic acid) to retard hydrolysis. Samples were partitioned into an organic phase (acetonitrile) and aqueous phase by C-18 SPE. Radioactivity was determined by LSC and the organic phase was subjected to chromatographic analysis (HPLC-RAM, TLC, LC-MS). The aqueous phase for the final sampling point and selected sampling points of B-ring incubations was acidified (HCl) and subjected to PLS-2 SPE. Radioactivity in organic and aqueous phases was determined by LSC. The resulting organic phase (acetonitrile) was subjected to chromatographic analysis (HPLC-RAM). Metabolite identification was obtained by comparison of retention times (HPLC), running factors (TLC) and MS spectra (LC-MS iontrap) with reference standards of metabolites (A1, B1, AB-1, A-18). Metabolites for which no references were available were identified by LC-MS (iontrap). Hydrolytic half-lives for cyflumetofen were calculated by linear regression using first order kinetics.

Results and Discussion

Mass balances were between 94.15 and 103.47% of applied radioactivity (AR) for [A-ring-U-14C]Cyflumetofen (all pH values) and between 97.62 and 102.15% AR for [B-ring-U-14C]Cyflumetofen (all pH values).

A-label incubation solutions partitioned nearly complete to the organic phase, whereas for the B-label incubations, radioactivity was equally distributed between organic and aqueous phases at the end of the incubation periods.

Hydrolysis (25°C) of cyflumetofen occurred over the entire pH range 4-9. Hydrolysis was most rapid at pH 9 (DT_{50} 10.3 min) and decreased with decreasing pH values (9.8 hrs at pH 7, 6 days at pH 5 and 7.7 days at pH 4, see

Table 125).

Table 125 Kinetic parameters for hydrolysis of cyflumetofen in aqueous buffer solutions of pH 4, 5, 7 and 9 at 25 °C.

Test	DT_{50}	DT_{90}	first order equation	r^2
pH4 [A-ring-U-14C] Cyflumetofen	7.7 d	25.6 d	lnC = -0.09008t + ln(95.0235)	0.9991
[B-ring-U-14C] Cyflumetofen	7.7 d	25.6 d	lnC = -0.08980t + ln(94.9601)	0.9977
Mean	7.7 d	25.6 d		
pH 5 [A-ring-U-14C]	6.0 d	20.0 d	lnC = -0.11504t + ln(97.4473)	0.9988

Cyflumetofen [B-ring-U-14C] Cyflumetofen	6.0 d	20.0 d	lnC = -0.11496t + ln(96.5132)	0.9994
Mean	6.0 d	20.0 d		
pH 7 [A-ring-U-14C] Cyflumetofen	10.3 hrs	34.1 hrs	lnC = -0.06751 + ln(94.9138)	0.9984
[B-ring-U-14C] Cyflumetofen	9.4 hrs	31.1 hrs	lnC = -0.07395t + ln(99.7485)	0.9997
Mean	9.8 hrs	32.6 hrs		
pH9 [A-ring-U-14C] Cyflumetofen	11.5 min	38.0 min	lnC = -0.06053t + ln(88.0009)	0.9428
[B-ring-U-14C] Cyflumetofen	9.1 min	30.4 min	lnC = -0.07583t + ln(91.9483)	0.9918
Mean	10.3 min	34.2 min		

Hydrolysis products exceeding 10% of applied radioactivity at environmentally relevant pH values (pH 5-7) were:

Table 126 Hydrolysis products exceeding 10% of applied radioactivity (pH 5-7)

	Max (% AR)	At time	remark
A-1	14.44	8 h	thereafter, the concentration declined to n.d.
A-2	44.12	720 h	max reached at the end of the measurement period (720 h)
A-18	36.22	120	decline to 10.85 %AR at the end of the measurement periode (720 h)
AB-1	44.51	120	decline to 36.96 %AR at the end of the measurement periode (720 h)
B-1	53.17	48 h	no significant decline upto the end of the measurement periode (720 h)

Unknown fractions were all ≤7.76% AR, with the exception of one hydrolysis product at day 14: 11.39% AR (pH 5, B-label). When taking the average of the A- and B-label incubations at pH 5, also this fraction was <10% AR at all time points.

Structures and the hydrolysis pathway are given in Figure 2

Conclusions

Cyflumetofen is susceptible to hydrolysis in acidic, neutral and basic buffer solutions. The hydrolysis rate increases with increasing pH. Hydrolytic half-lives of cyflumetofen at 25 °C are 7.7 d (pH 4), 6.0 d (pH 5), 9.8 hrs (pH 7) and 10.3 min (pH 9). Hydrolysis could be described by first order kinetics (r^2 0.9428-0.9997).

At environmentally most relevant pH values, hydrolysis products exceeding 10% of applied radioactivity were A-1 (max 14.44% AR at pH 5-7), A-2 (max 44.12% AR at pH 5-7), A-18 (max 36.22% AR at pH 5-7), AB-1 (max 44.51% AR at pH 5-7) and B-1 (max 53.17% AR at pH 5-7).

Unknown fractions were all ≤7.76% AR, with the exception of one hydrolysis product at day 14: 11.39% AR (pH 5, B-label). When taking the average of the A- and B-label incubations at pH 5, also this fraction was <10% AR at all time points.
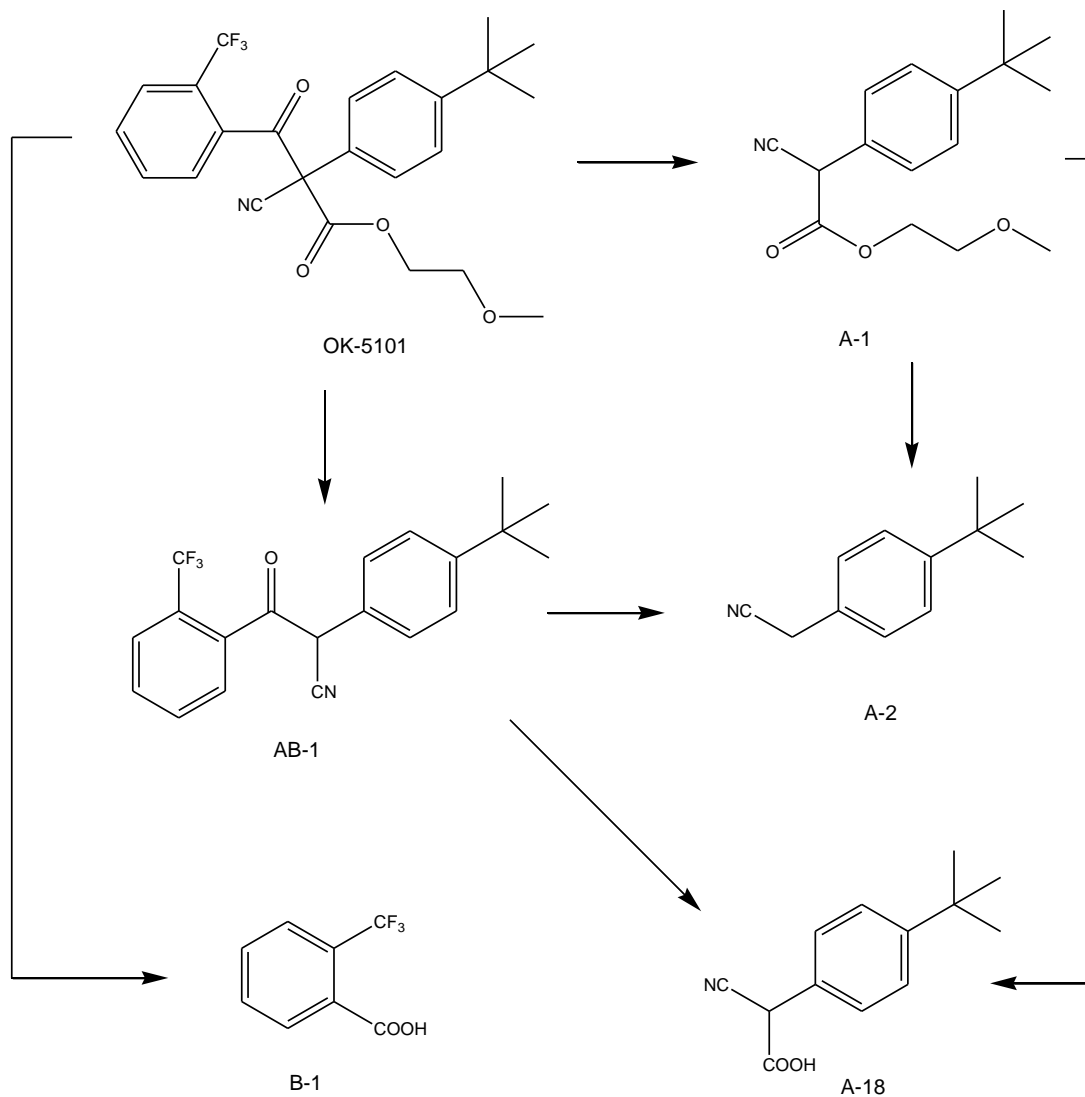
Figure 2   Hydrolysis pathway of cyflumetofen (in above figure indicated as OK-5101)

Aquatic photolysis

reference	:	Ohyama K. (2004d)	incubation time	:	48 hours	
study type	:	aqueous photolysis	nominal concentration	:	0.01 µg/mL	
year of execution	:	2004	pH/temperature	:	pH 5/temp 25°C	
GLP statement	:	Yes	light intensity	:	20 W/m^2 at 290-400 nm	
guideline	:	JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993)	Conclusion	:	Photolytic DT$_{50}$ under study conditions (continuous irradiation with Xenon light, 3 mW/cm^2) 1.28 hr at pH 5, 1.07 hr in natural water, quantum yield 3.0x10^{-5} mole/Einstein.	
test substance	:	[A-ring-U-^{14}C]Cyflumetofen (batch CFQ12735) [B-ring-U-^{14}C]Cyflumetofen (batch CFQ12736)	acceptability	:	Acceptable	
purity	:	Chemical purity not reported, radiochemical purity 99.2 (A-ring), 98.1% (B-ring).				

Portions of 4 mL [A-ring-U-^{14}C]Cyflumetofen or [B-ring-U-^{14}C] Cyflumetofen in acetonitrile were spiked to 400 mL sterilised buffer solutions of pH 5 (0.2M Acetic acid/sodium acetate) and to 400 mL natural water samples (Kokaigawa river, Ibaraki, Japan (1.2 µm filtered)) in quartz vessels at a concentration of 0.01 mg/L. Radiopurity and concentration of the spike solution were determined by HPLC and LSC, respectively. The light exposed test samples were irradiated (20 W/m^2 at 290-400 nm) by a Xenon light at 25±1°C. The Xenon light was equipped with a UV-filter (cut-off 290 nm) and IR filter (cut-off 800 nm). Temperature was monitored and light intensity was measured at the start and end of the experiment. Dark control samples were incubated at 25±1°C. Sterility was checked at the beginning and end of the experiment. Irradiation and dark incubation took place over a period of 48 hours. Samples were taken at various time points (0min, 20min, 40min, 1h, 2h, 4h and 2d). Immediately after sampling, the samples were acidified (1 mL acetic acid). Samples were partitioned into an organic phase (acetonitrile eluate) and aqueous phase by C-18 SPE. Radioactivity was determined by LSC and the organic phase was subjected to chromatographic analysis (HPLC-RAM, LC-MS). The aqueous phase (when > 5% applied radioactivity) was acidified (HCl) and subjected to C-18 SPE. Radioactivity in organic and aqueous phases was determined by LSC. The resulting organic phase (acetonitrile) was subjected to chromatographic analysis (HPLC-RAM). The method was validated for cyflumetofen (recoveries 95.5-97.6%). Metabolite identification was obtained by comparison of retention times (HPLC) and MS spectra (LC-MS iontrap) with reference standards of metabolites (A-1, A-2, A-12, A-14, A-18, B-1, AB-1, AB-6 and AB-7). Metabolites for which no references were available were identified by LC-MS (iontrap). Half-lives for cyflumetofen were calculated by linear regression using first order kinetics.

Results and Discussion

The radiochemical purity of the application solution was >98%. Actual test solution concentrations of cyflumetofen were 0.0097-0.0098 mg/L. The measured light intensity was 20 W/m^2 (290-400 nm), 19.96 W/m^2 (300-400 nm) and 179.9 W/m^2 (290-800 nm). Sterility of the test solutions was confirmed.

Mass balances were between 95.57 and 103.63% of applied radioactivity (AR) for both labels, dark and irradiated, pH 5 and natural water.

A-label incubation solutions partitioned nearly complete to the organic phase, whereas for the B-label incubations, some radioactivity was observed in the aqueous phase (max 5.47% AR).

Cyflumetofen was susceptible to aquatic photolysis in both pH 5 buffer solutions and in natural water. In irradiated (20 W/m^2 290-400 nm) buffer pH 5 the mean DT$_{50}$ was 1.28 hours, whereas under dark conditions it was 134 hours. In irradiated natural water, the DT$_{50}$ was slightly lower compared to irradiated pH 5 buffer: 1.07 hr (vs 1.28 hr). In natural dark water the DT$_{50}$ was much lower compared to dark pH 5 buffer: 3.40 hr (vs. 134 hours), caused by increased hydrolysis at the pH (7.48) of natural water. All DT$_{50}$ values were based on single first order kinetics (SFO) kinetics. Details are provided in Table 127.

Table 127   SFO kinetic parameters for aquatic photolysis of cyflumetofen in aqueous buffer solution of pH 5 and in natural water (25 °C).

Test	DT$_{50}$ (hr) (SFO)		DT$_{90}$ (hr) (SFO)		r^2 (SFO)	
	Light	Dark	Light	Dark	Light	Dark
pH 5 buffer [A-ring-U-14C]Cyflumetofen	1.17	135.09	3.90	448.76	1.00	0.90
[B-ring-U-14C]Cyflumetofen	1.39	133.85	4.61	444.64	0.99	0.97
Mean	1.28	134.47	4.26	446.70	-	-
natural water [A-ring-U-14C]Cyflumetofen	0.96	3.61	3.19	12.00	1.00	1.00
[B-ring-U-14C]Cyflumetofen	1.17	3.19	3.90	10.60	0.97	0.99
Mean	1.07	3.40	3.55	11.30	-	-

Aquatic photolysis products (in buffer pH 5) exceeding 10% of applied radioactivity were:

Table 128 Aquatic photolysis products exceeding 10% of applied radioactivity (pH 5)

	Max (% AR)	At time	Remarks
B-1	11.88 (13.27)[a]	48 h	max reached at the end of the measurement period (48 h)
AB-7	10.82	4 h	decline to 5.73 %AR at the end of the measurement periode (48 h)
AB-15	54.67	48 h	max reached at the end of the measurement period (48 h)

[a] under dark conditions

In natural water, AB-1 was also observed in significant amounts (max 43.77% AR under dark and 12.45% AR under irradiated conditions). Minor metabolites detected at pH 5 and in natural water were A-1, A-2, A-12, A-14, A-18, AB-1 and AB-6, all ≤6.22% AR (pH 5) and ≤9.67 (natural water). In summary, the only specific photolytic degradation products of cyflumetofen are AB-15 and AB-7. Structures and the photolysis pathway are given in Figure 3.

Conclusions

Cyflumetofen is susceptible to aquatic photolysis in aqueous buffer solution (pH 5). Cyflumetofen degraded with a half-life of 1.28 hr under irradiated (20 W/m^2, 290-400 nm) conditions and 134.47 hr under dark conditions. In irradiated natural water, the DT$_{50}$ was 1.07 hours.

Aquatic photolysis products (in buffer pH 5) exceeding 10% of applied radioactivity were B-1 (max 11.88% AR), AB-7 (max 10.82% AR) and AB-15 (max 54.67% AR). No other degradation products above 10% AR were observed. B-1 was also a significant degradation product under dark conditions (max 13.27 % AR). In natural water, AB-1 was also observed in significant amounts

(max 43.77% AR under dark and 12.45% AR under irradiated conditions). In summary, the only specific photolytic degradation products of cyflumetofen are AB-15 and AB-7.
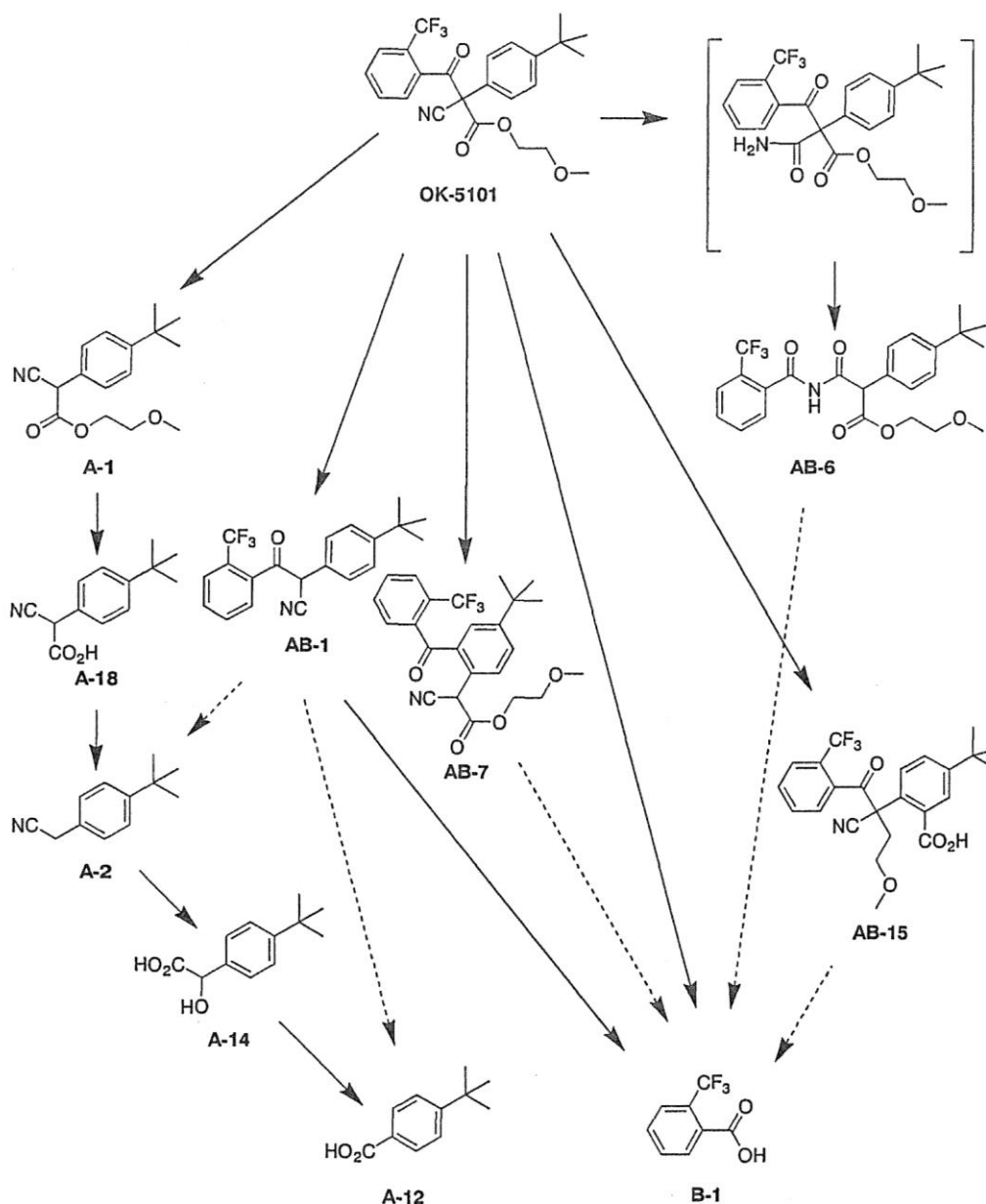
Figure 3   Aquatic photolysis pathway of cyflumetofen (indicated as OK-5101 in the figure)

Air

The overall half-life for cyflumetofen degradation in air (reaction with OH radicals) was firstly calculated based on a 12-h OH-radical concentration of 9.7×10^5 molecules cm^{-3} (Atkinson calculation). This was subsequently recalculated by the Rapporteur Member State based on 1.5×10^6 molecules cm^{-3}, resulting in a DT_{50} value of 8.2 hours (0.34 day).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

There is no estimation for biodegradability available.

5.1.2.2 Screening tests

There is no screening test for biodegradability available.

5.1.2.3 Simulation tests

Two water/sediment studies were performed with a ^{14}C label in either of the two aromatic rings of cyflumetofen (A-ring and B-ring).

^{14}C label in the A-ring

Reference	:	Noorloos van B. and Brands C. (2008)	incubation time	:	57 (Schoonrewoerdse Wiel) and 98 (Goorven) days
year of execution	:	2006-2008	nominal concentration	:	~0.30 mg/L
GLP statement	:	Yes	temperature	:	19.1-21.2°C
Guideline	:	OECD 308	DT_{50}	:	see results
test substance	:	A-[ring-U-^{14}C] Cyflumetofen (batch CFQ12735)	metabolites	:	see results
purity	:	Chemical purity not reported, radiochemical purity >97%	acceptability	:	Acceptable
test system	:	sand and silty clay loam/silt loam sediment			

The fate of [A-ring-U-^{14}C]Cyflumetofen was studied in an OECD Test Guideline 308 compliant test in two different water/sediment systems used within 4 days of field sampling (storage 4°C). Water (180 µm) and sediment (2 mm) were sieved prior to use. The water/sediment properties are listed in Table 129.

Table 129 Properties of water/sediment systems

Property	Goorven (GV)	Schoonrewoerdsewiel (SW)
Origin	Oisterwijk, The Netherlands	Schoonrewoerd, The Netherlands
Sediment		
Texture (USDA)	Sand	Silty clay loam / silt loam
Sand (%, USDA)	98	16
Silt (%, USDA)	1	57
Clay (%, USDA)	1	27
pH-water	-	-
pH-KCl	4.56-5.13	7.17-7.23
pH-CaCl$_2$	-	-
% OM	1.40	10.7
% OC	0.81	6.2
CEC (meq/100g)	0.7	31.5
Water		
pH	6.2	4.87
TOC (mg/L)	20.8-69.3	8.6-22.9
Total N (mg/L)	1.7	1.1
Total P (mg/L)	<0.3	<0.3
Hardness (mg/L CaCO$_3$)	122	196

Water/sediment systems with a sediment layer of ~2 cm and a water layer of ~6 cm were prepared in glass metabolism flasks. The flasks were placed in a climatised room (~20°C) and connected to an air stream, which was allowed to bubble gently through the upper layer of the water layer intermittently during the entire equilibration period. Aeration took place twice daily for 30 minutes. During equilibration, dissolved oxygen, pH, and redox potential were determined weekly in the water layer of two dedicated flasks for each system. The redox potential in the sediment layer was determined once in the same two flasks for each system. Equilibration lasted for 9 and 20 days. At the end of the equilibration period, microbial biomass in the sediment layer was determined using the fumigation-extraction method.

Following equilibration, [A-ring-U-^{14}C]Cyflumetofen was spiked (acetonitrile, max 0.1% in water layer) to the water layer at a concentration of ~0.3 mg/L. Immediately after spiking, the metabolism flasks were placed in a climatised room (~20°) in the dark and connected to a series of traps: (1) a polyurethane foam plug inserted in the neck of the metabolism flask, (2) a liquid trap containing ethylene glycol monoethyl ether and (3) two liquid traps containing 2N NaOH. During incubation, aeration took place twice a day for thirty minutes. The ingoing air was allowed to bubble gently – in order not to disturb the sediment layer - through the upper part of the water layer before leaving the metabolism flasks.

The incubation lasted 98 days for the Goorven system and 57 days for the Schoonrewoerdsewiel system. Incubation took place in the dark at 20 ± 2°C. The temperature in the climatised room was continuously monitored. During incubation, dissolved oxygen, pH, and redox potential were determined once a week in the water layer. The redox potential in the sediment layer was determined just prior to each sampling event.

Two metabolism flasks of each system were harvested at t=0 (30 minutes after spiking; single sample), 1, 3, 8, 15, 30, 59 and 98 days after spiking for the GV system and at t=0 (30 minutes after spiking; single sample), 2 and 16 hours, and 2, 5, 12, 29 and 57 days after spiking for the SW system. At sampling, also the polyurethane foam plugs and liquid traps were analysed. Liquid traps were also analyzed and replaced after 17 (GV), 29 (SW) and 59 (GV) days to avoid saturation of the traps.

The water layer was carefully decanted from the metabolism flask in a weighed glass container over a paper filter under vacuum. The SW water was then brought to pH 4 by addition of acetic acid. The pH of the GV water was measured and, if necessary, also brought to pH 4 using acetic acid. A weighed sub-sample partitioned with dichloromethane (4x). The dichloromethane extracts were concentrated and re-dissolved in acetonitrile. When the aqueous residue contained >5% of the applied radioactivity, it was subjected to freeze-drying. After freeze-drying, the residue was reconstituted in acetonitrile:Milli-Q water 1:1 (v/v). Radioactivity was determined by LSC in final and intermediate fractions.

The sediment layer was extracted (4x) with acetonitrile:sodium acetate buffer pH 4 3:1 (v/v). The extracts were concentrated and partitioned four times with dichloromethane. The dichloromethane extracts were concentrated and re-dissolved in acetonitrile. When the aqueous residue contained >5% of the applied radioactivity, it was subjected to freeze-drying. After freeze-drying, the residue was reconstituted in acetonitrile:Milli-Q water 1:1 (v/v). Radioactivity was determined by LSC in final and intermediate fractions. Residual activity in the sediment was determined by combustion/LSC. When the sediment contained >10% of applied after extraction (based on combustion/LSC), a sub-sample was transferred to a Soxhlet apparatus for Soxhlet extraction with acetonitrile for 3 hours.

Polyurethane foam (PUF) plugs were extracted with acetonitrile. Total radioactivity in the liquid traps was determined by LSC.

Analysis of extracts was performed by reversed phase HPLC-RAM and TLC. Identification was by co-chromatography, GC-MS and LC-MS/MS analysis.

The DT_{50} and DT_{90} values were calculated using the amounts of cyflumetofen as determined by HPLC.

Optimisations were performed using the program ModelMaker v 4.0. To obtain endpoints for assessment of persistence, the parent data (not averaged) in the water layer, in the sediment and in the total system were fitted to single first order kinetics (SFO) and to the Gustafson and Holden model (FOMC).

For calculation of the half-life of metabolites (GV Met-5 and Met-7, SW Met-8 and Met-13), the data were fitted to SFO kinetics (decline from maximum observed % onwards).

Results and Discussion

During the total equilibration and incubation period, the temperature was within the range 19.1-21.2°C.

At the start of the equilibration period, microbial biomass was 0.2% for the Goorven system and 2.8% for the Schoonrewoerdsewiel system. At the end of the incubation period, microbial biomass was 0.4% for the Goorven system and 1.8% for the Schoonrewoerdsewiel system. The results indicate sufficiently viable conditions for the SW system. For GV the biomass was low at the start, but increased during the study. CO_2 production (mineralisation) also continued throughout the study, indicating sufficiently viable conditions.

The dissolved oxygen concentrations in the water layer fluctuated between 7.2 and 9.2 mg/L (GV) and 4.8 and 8.4 mg/L (SW). Together with the positive redox potentials (195 to 433 mV (GV) and 139 to 279 mV (SW)), the measurements indicate aerobic conditions in the water layer. Redox potentials in the sediment varied from –246 to -113 mV (GV) and from -314 to -49 mV (SW) indicating anaerobic conditions.

Detailed results are given in Table 130, Table 131, Table 132, Table 133. The proposed degradation pathway of A-ring labelled cyflumetofen in water/sediment systems is given in Figure 4.

Goorven system

The mass balances (mean values of duplicates) for the Goorven system ranged from 89 to 97% of applied radioactivity, except after 30 and 59 days (74-76% of applied).

Only very small amounts of radioactivity (≤0.7% of applied) were found in the polyurethane foam plugs and the ethylene glycol monoethyl ether traps. Approximately 20% of applied was recovered as CO_2 at the end of the test.

The amount of radioactivity recovered in the water layer decreased to 19% of applied after 8 days and remained fairly constant afterwards (21% after 98 days).

Total radioactivity in the sediment layer reached a maximum of 72% of applied radioactivity after 8 days of incubation and decreased to 48% after 98 days. Up to 59% of the applied radioactivity (after 3 days) could be extracted from the sediment. Extractable radioactivity decreased to 15% of applied

at the end of the study. Soxhlet extraction released only 0.6-2.5% of applied. Bound residues increased slowly to maximum 33% of applied radioactivity after 98 days.

The (mean) amount of Cyflumetofen in the water layer of the Goorven system decreased from 86% to 1.9% of applied radioactivity within 15 days and Cyflumetofen had fully dissipated from the water layer from day 59 onwards. Up to five metabolite fractions were observed in the water layer. One major metabolite (>10%) was observed: Met-1 (maximum 10.7% of applied after 59 days). Met-1 did not match any available metabolite standards. Based on LC-MS/MS the following structure was proposed for Met-1:
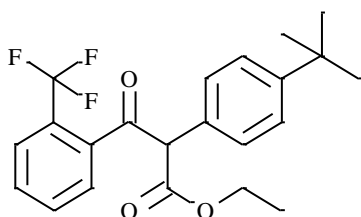
Cyflumetofen in the sediment extract(s) increased to maximum 54% of applied after 3 days, and then decreased to 2.4% after 98 days. Up to 7 metabolites were detected, none of them exceeded 10% of applied.

Schoonrewoerdsewiel system

The mass balances (mean values of duplicates) for the Schoonrewoerdsewiel system ranged from 91 to 100% of applied radioactivity.

Only very small amounts of radioactivity (≤1.8% of applied) were found in the polyurethane foam plugs and the ethylene glycol monoethyl ether traps. Approximately 2% of applied was recovered as CO_2 at the end of the test.

The amount of radioactivity recovered in the water layer decreased to 57% of applied after 0.1 day (2 hours), but increased again to 85% after 0.7 day (16 hours) and decreased again to 25% after 57 days. It is expected that Cyflumetofen adsorbs to the sediment and then degrades to metabolites that desorb again, which explains the sequential decrease-increase-decrease of radioactivity in the water layer.

Total radioactivity in the sediment layer was 34% of applied radioactivity after 2 hours, decreased to 16% after 16 hours, and then gradually increased to 66% of applied after 57 days. The lower percentage radioactivity in the sediment at t=16 hours is expected to be due to desorption of metabolites. This corresponds to the increased levels in the water layer. Up to 50% of applied radioactivity (after 29 days) could be extracted from the sediment (49% after 57 days). Soxhlet extraction released only 1.4-2.9% of applied. Bound residues increased slowly to maximum 17% of applied radioactivity at the end of incubation.

The (mean) amount of Cyflumetofen in the water layer of the Schoonrewoerdsewiel system decreased from 91.5% to 0.8% of applied radioactivity within 2 days and Cyflumetofen had fully dissipated from the water layer from day 5 onwards (except one detect at day 57 of 2%). Up to 14 metabolite fractions were observed in the water layer, three of which exceeded 10% of applied:

Met-5 (maximum 18.4% of applied after 0.7 days), Met-8 (maximum 19.5% of applied after 5 days) and Met-10 (maximum 10% of applied after 0.7 days). Based on HPLC retention time, Met-5 matched with A-2/A-18 (A-2 confirmed by GC-MS), Met-8 remained unidentified and Met-10 matched with AB-11. Despite further attempts (LC-MS/MS), Met-8 could not be identified.

Cyflumetofen in the sediment extract increased to maximum 28% of applied after 2 hours, dropped to 4.4% after 16 hours, increased again to 11% after 2 days and then decreased to 1.2% after 57 days. Up to 12 metabolites were detected, of which three metabolites exceeded 10% of applied: Met 4 (maximum 10.7% after 29 days), Met-7 (maximum 14.6% after 29 days) and Met-10 (maximum 10.1% after 12 days). Based on HPLC retention time, Met-7 matched with AB-1 (confirmed by GC-MS), Met-4 remained unidentified and Met-10 matched with AB-11. Despite further attempts (LC-MS/MS), Met-4 could not be identified.

Table 130 Identification of radioactivity in Goorven water layer (% of AR)

Time (days)	Cyflumetofen 14.1-15.9 min	Met-1 2.4-2.6 min	Met-5[1] 9.8-10.9 min	Met-7[2] 11.3-12.3 min	Others[3]
0	86.0	0.0	0.0	0.0	0.0
1	34.6	0.0	1.4	0.0	1.5
3	23.4	0.0	1.5	0.0	1.7
8	5.9	0.0	2.5	0.0	1.8
15	1.9	1.7	9.3	2.0	1.2
30	2.0	1.3	4.3	0.0	3.1
59	0.0	10.7	0.0	0.0	1.3
98[4]	0.0	0.0	0.0	0.0	0.0

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied. Percentages are based on the radioactivity in the concentrated extracts.

[1] Match with A-18 and A-2 based on retention time. The presence of A-2 was confirmed by GC-MS.

[2] Match with AB-1 based on retention time. The presence of AB-1 was confirmed by GC-MS.

[3] Each individual peak <5% of applied.

[4] Diffuse radioactivity. No distinct peaks in chromatogram.

Table 131 Identification of radioactivity in Schoonrewoerdsewiel water layer (% of AR)

Time (days)	Cyflumetofen 14.4-16.0 min	Met-1 2.4 min	Met-4 6.2-8.8 min	Met-5[1] 9.1-10.9 min	Met-7[2] 11.8-12.7 min	Met-8 13.1-14.0 min	Met-10[3] 16.2-18.5 min	Met-13[4] 20.0-22.0 min	Met-14 20.7-22.5 min	Others[5]
0	91.5	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
0.1	37.0	0.0	0.0	1.0	0.7	2.9	1.8	1.3	1.3	7.6
0.7	7.1	0.0	4.9	18.4	6.4	9.2	10.0	5.3	5.5	4.9
2	0.8	0.0	2.5	13.7	0.0	4.7	4.7	7.6	6.6	11.6
5	0.0	0.0	2.9	17.7	0.0	19.5	1.5	0.3	0.5	3.1
12	0.0	5.8	2.0	3.9	0.0	3.3	1.0	1.0	0.5	1.7
29	0.0	7.5	7.6	6.0	0.5	6.2	0.0	0.0	0.0	4.3
57	2.0	0.0	6.8	5.2	0.0	0.0	0.0	0.2	0.0	3.7

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied. Percentages are based on the radioactivity in the concentrated extracts.

[1] Match with A-18 and A-2 based on retention time. The presence of A-2 was confirmed by GC-MS.

[2] Match with AB-1 based on retention time. The presence of AB-1 was confirmed by GC-MS.

[3] Match with AB-11 based on retention time.

[4] Match with AB-12 based on retention time.

[5] Each individual peak <5% of applied.

Table 132 Identification of radioactivity in Goorven sediment layer (% of AR)

Time (days)	Cyflumetofen 14.1-15.9 min	Met-1 2.4-2.6 min	Met-5[1] 9.8-10.9 min	Met-7[2] 11.3-12.3 min	Others[3]
0	Na	na	na	na	Na
1	43.9	0.0	0.0	0.4	0.0
3	54.3	0.0	0.2	1.1	0.5
8	46.6	0.0	1.0	1.7	3.9
15	24.9	0.0	2.7	4.6	4.3
30	14.6	0.0	0.6	2.3	6.6
59	5.8	0.0	0.6	2.4	6.0
98	2.4	0.0	1.9	0.0	6.0

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied. Percentages are based on the radioactivity in the concentrated extracts.

[1] Match with A-18 and A-2 based on retention time. The presence of A-2 was confirmed by GC-MS.

[2] Match with AB-1 based on retention time. The presence of AB-1 was confirmed by GC-MS.

[3] Each individual peak <5% of applied.

na: not applicable

Table 133 Identification of radioactivity in Schoonrewoerdsewiel sediment layer (% of AR)

Time (days)	Cyflumetofen 14.4-16.0 min	Met-1 2.4 min	Met-4 6.2-8.8 min	Met-5[1] 9.1-10.9 min	Met-7[2] 11.8-12.7 min	Met-8 13.1-14.0 min	Met-10[3] 16.2-18.5 min	Met-13[4] 20.0-22.0 min	Met----14 20.7-22.5 min	Others[5]
0	Na	na	na	na	na	na	na	na	na	na
0.1	28.3	0.0	0.0	0.7	0.5	0.0	0.7	0.0	0.0	0.0
0.7	4.4	0.0	0.0	0.5	1.4	0.0	3.7	0.0	0.0	0.3
2	11.1	0.0	1.8	2.4	3.8	0.0	7.7	0.0	0.0	0.0
5	4.1	0.0	2.4	5.0	9.0	0.0	8.9	0.0	0.0	0.5
12	0.3	0.0	6.0	2.0	9.8	0.0	10.1	1.4	2.4	3.3
29	0.0	0.0	10.7	1.6	14.6	2.3	7.4	0.0	1.1	4.0
57	1.2	0.0	5.4	6.1	2.4	0.0	5.9	1.7	3.9	11.3

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied. Percentages are based on the radioactivity in the concentrated extracts.

[1] Match with A-18 and A-2 based on retention time. The presence of A-2 was confirmed by GC-MS.

[2] Match with AB-1 based on retention time. The presence of AB-1 was confirmed by GC-MS.

[3] Match with AB-11 based on retention time.

[4] Match with AB-12 based on retention time.

[5] Each individual peak <5% of applied

na: not applicable

Estimated DT_{50} and DT_{90} of cyflumetofen and relevant metabolites are given in Table 134, Table 135 and Table 136.

Table 134 Half-lives for dissipation/degradation of cyflumetofen in Goorven water/sediment system.

Compart-ment	parameter for	kinetics	quality of fit	χ^2 (err)	r^2	DT_{50} (days)	DT_{90} (days)
Water	persistence	FOMC	best fit	9.96	0.98	0.7	7.5
Sediment	persistence	FOMC	best fit	9.35	0.98	12.0	50.6
Total system	persistence	HS	best fit	6.61	0.99	9.9	48.7

[1] Best fit, SFO not acceptable

Table 135 Half-lives for dissipation/degradation of cyflumetofen in Schoonrewoerdsewiel water/sediment system.

Compart-ment	parameter for	kinetics	quality of fit	χ^2 (err)	r^2	DT$_{50}$ (days)	DT$_{90}$ (days)
Water	persistence	FOMC	best fit	2.40	0.99	0.1	0.5
Sediment	persistence	SFO	best fit	13.38	0.97	2.1	7.0
Total system	persistence	HS	best fit	5.83	0.99	0.2	2.2

Table 136 Half-lives for dissipation/degradation of A-2, AB-1, Met-8 and Met-13 in water/sediment systems

System	Metabolite	Degradation rate (day^{-1})	χ^2 (err)	r^2	DT$_{50}$ (days)	DT$_{90}$ (days)	Data included (days)
GV	A-2	0.059 ± 0.032	15.40	0.6677	11.74	39.01	15-98
GV	AB-1	0.038 ± 0.014	23.75	0.7151	18.29	60.75	15-59
SW	Met-8	0.224 ± 0.175	41.15	0.4151	3.09	10.27	5-29

Compartment: total system   Kinetics: SFO.

Conclusions

Upon addition of cyflumetofen to the water layer, cyflumetofen is partitioned between the water and sediment layer. Radioactivity in the GV water layer decreased to ca. 19% of applied after 8 days and remained fairly constant afterwards. Radioactivity in the SW water layer decreased to ca. 57% of applied and then increased again to 85% followed by a decrease to 25% at the end of incubation. Mineralisation to CO_2 was a minor process in SW (2% after 57 days) but was significant in GV (20% after 98 days). Bound residues accounted for maximum 33 (GV) and 17% (SW) of applied.

In both systems, several relevant metabolites/metabolite fractions (>10% of applied) were formed (water: Met-1 (max 10.7%, 59d), A-2 (max 18.4%, 0.7 d), Met-8 (max 19.5%, 5 d) and AB-11 (max 10%, 0.7d); sediment: Met-4 (max 10.7%, 29 d), AB-1 (max 14.6%, 29 d) and AB-11 (max 10.1%, 12 d)). For Met-1 a structure was proposed. Met-4 and 8 remained unidentified (but as they are not observed in the B-label study *(see next study)*, they contain only the A-label ring). See Figure 4 for proposed degradation pathway.

The DT$_{50}$ values (persistence) for degradation of cyflumetofen from the total system were 0.2 (SW, HS kinetics) and 9.9 days (GV, HS kinetics). DT$_{90}$ values were 2.2 (SW) and 48.7 days (GV).

OK-5101

AB-1

AB-11

Met 1

A-2

minor transformation products, CO_2, bound residues
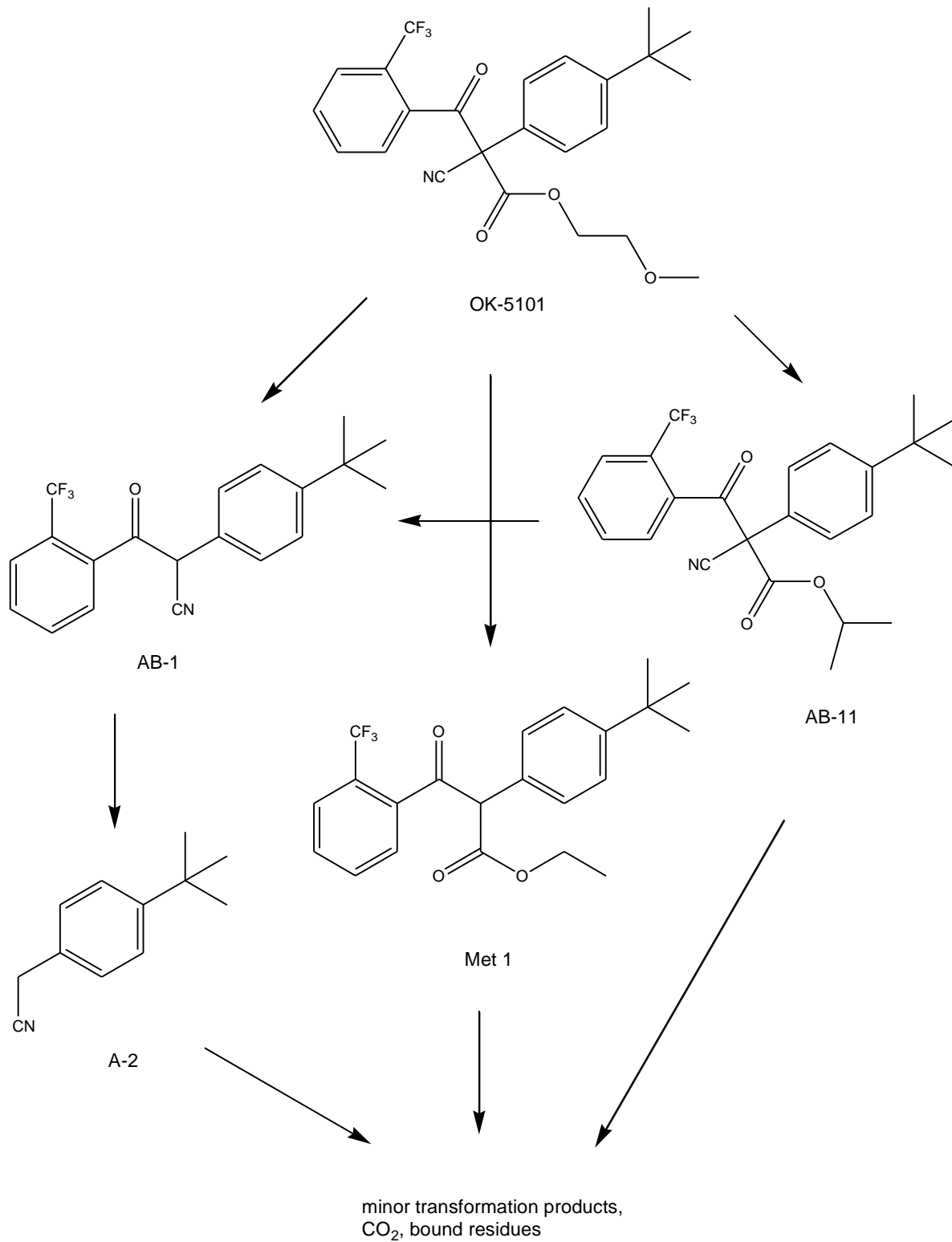
Figure 4 Degradation pathway of cyflumetofen (indicated as OK-5101) in water-sediment (A-label)

^{14}C label in the B-ring

reference	:	Noorloos van B. and J. de Mol (2007)	incubation time	:	103 days	
year of execution	:	2006-2007	nominal concentration	:	~0.30 mg/L	
GLP statement	:	Yes	temperature	:	19.1-21.2°C	
guideline	:	OECD 308	DT_{50}	:	see results	
test substance	:	B-[ring-U-^{14}C] Cyflumetofen (batch CFQ12736)	metabolites	:	see results	
purity	:	Chemical purity not reported, radiochemical purity >97% .	acceptability	:	Acceptable	
test system	:	sand and silty clay loam/silt loam sediment				

The fate of B-[ring-U-^{14}C]Cyflumetofen was studied in two different water/sediment systems used within three weeks of field sampling (storage 4°C). Water (180 µm) and sediment (2 mm) were sieved prior to use. The water/sediment properties are listed in Table 137.

Table 137 Properties of water/sediment systems

Property	Goorven (GV)	Schoonrewoerdsewiel (SW)
Origin	Ooisterwijk, The Netherlands	Schoonrewoerd, The Netherlands
Sediment		
Texture (USDA)	Sand	Silty clay loam / silt loam
Sand (%, USDA)	98	16
Silt (%, USDA)	1	57
Clay (%, USDA)	1	27
pH-water	-	-
pH-KCl	4.56-5.54	7.17-7.36
pH-CaCl$_2$	-	-
% OM	1.40	10.7
% OC	0.81	6.2
CEC (meq/100g)	0.7	31.5
Water		
pH	6.2	4.87
TOC (mg/L)	20.8-51.7	8.6-21.8
Total N (mg/L)	1.7	1.1
Total P (mg/L)	<0.3	<0.3
Hardness (mg/L CaCO$_3$)	122	196

Water/sediment systems with a sediment layer of ~2 cm and a water layer of ~6 cm were prepared in glass metabolism flasks. The flasks were placed in a climatised room (~20°C) and connected to an air stream, which was allowed to bubble gently through the upper layer of the water layer intermittently during the entire equilibration period. Aeration took place twice daily for 30 minutes. During equilibration, dissolved oxygen, pH, and redox potential were determined weekly in the water layer of two dedicated flasks for each system. The redox potential in the sediment layer was determined once in the same two flasks for each system. The temperature in the climatised room was monitored continuously. Equilibration lasted for 15-16 days. At the end of the equilibration period, microbial biomass in the sediment layer was determined using the fumigation-extraction method.

Following equilibration, [B-ring-U-^{14}C]Cyflumetofen was spiked (acetonitrile, max 0.07% in water layer) to the water layer at a concentration of ~0.3 mg/L. Immediately after spiking, the metabolism flasks were placed in a climatised room (~20°) in the dark and connected to a series of traps: (1) a polyurethane foam plug inserted in the neck of the metabolism flask, (2) a liquid trap containing ethylene glycol monoethyl ether and (3) two liquid traps containing 2N NaOH. During incubation, aeration took place twice a day for thirty minutes. The ingoing air was allowed to bubble gently – in order not to disturb the sediment layer - through the upper part of the water layer before leaving the metabolism flasks.

The incubation lasted 103 days for both the Goorven system and the Schoonrewoerdsewiel system. Incubation took place in the dark at 20±2°C. The temperature in the climatised room was continuously monitored. During incubation, dissolved oxygen, pH, and redox potential were determined once a week in the water layer. The redox potential in the sediment layer was determined just prior to each sampling event.

Two metabolism flasks of each system were harvested at t=0 (30 minutes after spiking; single sample), 2, 5, 12, 29, 62 and 103 days after spiking for the GV system and at t=0 (30 minutes after spiking; single sample), 2 and 16 hours, and 2, 5, 12, 29 and 103 days after spiking for the SW system. At sampling, also the polyurethane foam plugs and liquid traps were analysed. Liquid traps were also analyzed and replaced after 28 days to avoid saturation of the traps.

The water layer was carefully decanted from the metabolism flask in a weighed glass container over a paper filter under vacuum. The SW water was then brought to pH 4 by addition of acetic acid. The pH of the GV water was measured and, if necessary, also brought to pH 4 using acetic acid. A weighed sub-sample partitioned with dichloromethane (4x). The dichloromethane extracts were concentrated and re-dissolved in acetonitrile. When the aqueous residue contained >5% of the applied radioactivity, it was subjected to freeze-drying. After freeze-drying, the residue was reconstituted in acetonitrile:Milli-Q water 1:1 (v/v). Radioactivity was determined by LSC in final and intermediate fractions.

The sediment layer was extracted (4x) with acetonitrile:sodium acetate buffer pH 4 3:1 (v/v). The extracts were concentrated and partitioned four times with dichloromethane. The dichloromethane extracts were concentrated and re-dissolved in acetonitrile. When the aqueous residue contained >5% of the applied radioactivity, it was subjected to freeze-drying. After freeze-drying, the residue was reconstituted in acetonitrile:Milli-Q water 1:1 (v/v). Radioactivity was determined by LSC in final and intermediate fractions. Residual activity in the sediment was determined by combustion/LSC. When the sediment contained >10% of applied after extraction (based on combustion/LSC), a sub-sample was transferred to a Soxhlet apparatus for Soxhlet extraction with acetonitrile for 3 hours.

Polyurethane foam (PUF) plugs were extracted with acetonitrile. Total radioactivity in the liquid traps was determined by LSC.

Analysis of extracts was performed by reversed phase HPLC-RAM and TLC. Identification was by co-chromatography, GC-MS and LC-MS/MS analysis.

The DT$_{50}$ and DT$_{90}$ values were calculated using the amounts of cyflumetofen as determined by HPLC. Choice of kinetic models was based on the FOCUS guidance document on estimating persistence and degradation kinetics.

Optimisations were performed using the program ModelMaker v 4.0. To obtain endpoints for assessment of persistence, the parent data (not averaged) in the water layer, in the sediment and in

the total system were fitted to single first order kinetics (SFO) and to the Gustafson and Holden model (FOMC).

For calculation of the half-life of metabolites (GV Met-5, SW Met-2 and Met-5), the data were fitted to SFO kinetics (decline from maximum observed % onwards).

Results and Discussion

During the total equilibration and incubation period, the temperature was within the range 19.1-21.2°C.

At the start of the equilibration period, microbial biomass was 0.04% for the Goorven system and 1.7% for the Schoonrewoerdsewiel system. At the end of the incubation period, microbial biomass was 1.9% for the Goorven system and 2.5% for the Schoonrewoerdsewiel system. The results indicate sufficiently viable conditions for the SW system. For GV the biomass was low at the start, but increased to acceptable levels during the study.

The dissolved oxygen concentrations in the water layer fluctuated between 6.1 and 8.3 mg/L (GV) and 5.8 and 9.2 mg/L (SW). Together with the positive redox potentials (238 to 411 mV (GV) and 130 to 238 mV (SW)), the measurements indicate aerobic conditions in the water layer. Redox potentials in the sediment varied from –228 to -68 mV (GV) and from -278 to -125 mV (SW) indicating anaerobic conditions.

Detailed results are given in Table 138, Table 139, Table 140 and Table 141. The proposed degradation pathway of B-ring labelled cyflumetofen in water/sediment systems is given in Figure 5.

Goorven system

The mass balances (mean values of duplicates) for the Goorven system ranged from 91 to 97% of applied radioactivity, except after 29 and 103 days (both 89% of applied).

Only very small amounts of radioactivity (≤0.7% of applied) were found in the polyurethane foam plugs and the ethylene glycol monoethyl ether traps. Approximately 3% of applied was recovered as CO_2 at the end of the test.

The amount of radioactivity recovered in the water layer decreased to 14% of applied after 5 days, but increased again to 27% after 12 days and to 56% after 103 days.

Total radioactivity in the sediment layer reached a maximum of 80% of applied radioactivity after 5 days of incubation and decreased to 30% after 103 days. Up to 73% of the applied radioactivity (after 5 days) could be extracted from the sediment. Extractable radioactivity decreased to 21% of applied at the end of the study. Soxhlet extraction released only 3% of applied. Bound residues increased slowly to maximum 19% of applied radioactivity after 62 days and decreased to 9.7% at the end of incubation.

The extracted Goorven sediment (T=29) contained 31.3% fulvic acids (3.9% of applied), 11.3% humic acids (1.4% of applied) and 43.4% humins (5.5% of applied).

The (mean) amount of cyflumetofen in the water layer of the Goorven system decreased from 93% to 1.4% of applied radioactivity within 12 days and cyflumetofen had fully dissipated from the water layer at the end of the incubation period. Up to five metabolite fractions were observed in the water layer. One major metabolite (>10%) was observed: Met-2 (maximum 51% of applied after 62

days). Based on HPLC retention times, Met-2 matched with B-1. This was confirmed by LC-MS analysis.

Cyflumetofen in the sediment extract(s) increased to maximum 67% of applied after 5 days, and then decreased to 1.5% after 103 days. Up to nine metabolites were detected, one of which exceeded 10% of applied: Met-5 (maximum 28% after 2 days). Based on HPLC retention time, Met-2 (<10%) matched with B-1 and Met-5 matched with AB-1 and B-2. [in the previously presented water-sediment study with A-label, AB-1 was also identified, indicating that Met-5 consisted of both AB-1 and B-2].

Schoonrewoerdsewiel system

The mass balances (mean values and individual samples) for the Schoonrewoerdsewiel system ranged from 95 to 102% of applied radioactivity, except at t=0 (115% of applied).

Only very small amounts of radioactivity (≤0.7% of applied) were found in the polyurethane foam plugs and the ethylene glycol monoethyl ether traps. Approximately 3% of applied was recovered as CO_2 at the end of the test.

The amount of radioactivity recovered in the water layer decreased to 75% of applied after 0.1 day (2 hours), but increased again to 95% after 0.7 day (16 hours) and decreased again to 50% after 103 days. It is expected that in both water/sediment systems, Cyflumetofen adsorbs to the sediment and then degrades to metabolites that desorb again, which explains the sequential decrease-increase-decrease of radioactivity in the water layer.

Total radioactivity in the sediment layer was 22% of applied radioactivity after 2 hours, decreased to 6.6% after 16 hours, and then gradually increased to 44% of applied after 103 days. The lower percentage radioactivity in the sediment at t=16 hours is expected to be due to desorption of metabolites. This corresponds to the increased levels in the water layer. Up to 33% of applied radioactivity (after 12 days) could be extracted from the sediment (31% after 103 days). Soxhlet extraction released only 3% of applied. Bound residues increased slowly to maximum 13% of applied radioactivity at the end of incubation.

The (mean) amount of cyflumetofen in the water layer of the Schoonrewoerdsewiel system decreased from 100% to 1.5% of applied radioactivity within 0.7 days and Cyflumetofen had fully dissipated from the water layer at the end of the incubation period. Up to seven metabolite fractions were observed in the water layer, two of which exceeded 10% of applied: Met-2 (maximum 65% of applied after 5 days) and Met-5 (maximum 15% of applied after 0.7 days). Based on HPLC retention time, Met-2 matched with B-1 and Met-5 matched with AB-1 and B-2. [in the water-sediment study with A-label, AB-1 was also identified, indicating that Met-5 consisted of both AB-1 and B-2].

Cyflumetofen in the sediment extract increased to maximum 20% of applied after 2 hours, dropped to 3.6% after 16 hours, increased again to 9.3% after 2 days and then decreased to 0.0% after 12 days. Up to ten metabolites were detected, of which the same metabolites as in the water layer exceeded 10% of applied: Met-2 (maximum 21% after 29 days) and Met-5 (maximum 11% after 12 days).

Table 138 Identification of radioactivity in Goorven water layer (% of AR)

Time (days)	Cyflumetofen 14.1-15.9 min	Met-2[1] 4.2-5.0 min	Others
0	93.1	0.0	0.0
2	9.0	22.2	1.2
5	6.5	5.7	0.0
12	1.4	23.7	0.0
29	0.0	36.8	0.7
62	0.0	50.9	1.2
103	0.0	42.6	1.6

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied.
[1] Match with B-1 based on retention time and MS spectrum.

Table 139 Identification of radioactivity in Schoonrewoerdsewiel water layer (% of AR)

Time (days)	Cyflumetofen 14.4-16.0 min	Met-2[1] 4.0-5.0 min	Met-5[2] 11.0-12.8 min	Others
0	100.2	0.0	0.0	0.0
0.1	25.6	29.6	10.5	0.0
0.7	1.5	64.2	15.4	1.6
2	0.0	54.8	0.0	0.0
5	0.0	65.0	0.0	0.7
12	0.0	63.5	0.0	0.0
29	0.0	52.9	0.0	0.0
103	0.0	49.1	0.0	0.3

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied.
Percentages are based on the radioactivity in the concentrated extracts.
[1] Match with B-1 on retention time. Match was confirmed by LC-MS.
[2] Match with AB-1 and B-2 based on retention time. The presence of B-2 was confirmed by GC-MS.

Table 140 Identification of radioactivity in Goorven sediment layer (% of AR)

Time (days)	Cyflumetofen 14.4-16.0 min	Met-2[1] 4.2-5.0 min	Met-5[2] 11.0-12.8 min	Others[3]
0	na	na	na	na
2	30.9	1.8	28.0	1.0
5	66.8	1.1	1.8	0.6
12	31.0	5.1	6.2	4.8
29	19.0	5.7	2.3	2.7
62	7.9	5.9	2.3	3.8
103	1.5	9.9	2.2	6.2

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied.
Percentages are based on the radioactivity in the concentrated extracts.
[1] Match with B-1 on retention time. Match was confirmed by LC-MS.
[2] Match with AB-1 and B-2 based on retention time. The presence of B-2 was confirmed by GC-MS.
[3] Each individual peak <5% of applied
na: not applicable

Table 141 Identification of radioactivity in Schoonrewoerdsewiel sediment layer (% of AR)

Time (days)	Cyflumetofen 14.4-16.0 min	Met-2[1] 4.0-5.0 min	Met-5[2] 11.0-12.8 min	Others

0	na	na	na	na
0.1	19.7	0.0	0.4	0.0
0.7	3.6	0.1	0.8	0.1
2	9.3	9.1	0.0	0.0
5	2.0	15.1	10.0	0.5
12	0.0	20.9	10.9	0.8
29	0.0	21.5	7.6	3.1
103	0.0	18.5	2.4	2.8

Aqueous residues of the water layer and the sediment extract were only included when they contained >5% of applied. Percentages are based on the radioactivity in the concentrated extracts.

[1] Match with B-1 on retention time. Match was confirmed by LC-MS.

[2] Match with AB-1 and B-2 based on retention time. The presence of B-2 was confirmed by GC-MS.

na: not applicable

Estimated DT_{50} and DT_{90} of cyflumetofen and relevant metabolites are given in Table 142, Table 143 and Table 144.

Table 142 Half-lives for dissipation/degradation of cyflumetofen in Goorven water/sediment system

Compart-ment	parameter for	kinetics	quality of fit	χ^2 (err)	r^2	DT50 (days)	DT90 (days)
Water	persistence	FOMC	best fit	3.86	1.00	0.1	2.0
Sediment	persistence	SFO	best fit	8.62	0.99	15	50
Total system	persistence	SFO	best fit	5.44	0.99	14	47

Table 143 Half-lives for dissipation/degradation of cyflumetofen in Schoonrewoerdsewiel water/sediment system

Compart-ment	parameter for	kinetics	quality of fit	χ^2 (err)	r^2	DT50 (days)	DT90 (days)
Water	persistence	SFO	best fit	1.77	0.99	0.05	0.15
Sediment	persistence	SFO	best fit	44.28	0.52	0.6	1.9
Total system	persistence	DFOP	best fit	6.89	0.98	0.08	0.4

Table 144 Half-lives for dissipation/degradation of B-1 and B-2 in water/sediment systems

System	Metabolite	Degradation rate (day^{-1})	χ^2 (err)	r^2	DT_{50} (days)	DT_{90} (days)	Data included (days)
GV	B-2	0.903	33.33	0.658	0.77	2.55	2-103
SW	B-1	0.00216	3.52	0.688	320	1065	12-103
SW	B-2	0.0175	3.40	0.959	40	131	12-103

Compartment: total system   Kinetics: SFO.

Conclusions

Upon addition of cyflumetofen to the water layer, cyflumetofen is partitioned between the water and sediment layer. Radioactivity in the GV water layer decreased to ca. 14% of applied and then increased again to 56% at the end of incubation. Radioactivity in the SW water layer decreased to ca. 75% of applied and then increased again to 95% followed by a decrease to 50% at the end of

incubation. Mineralisation to CO_2 was a minor process both systems (3% at the end of the study). Bound residues accounted for maximum 19 (GV) and 13% of applied.

In both systems, two relevant metabolites/metabolite fractions (>10% of applied) were formed (water: B-1 (max 65%, 5 d) and B-2 (max 15.4%, 0.7 d); sediment: B-1 (max 21.5% (29 d) and B-2 (max 28%, 2 d)).

Persistence DT_{50} values for degradation of cyflumetofen from the total system were 0.08 (SW, DFOP kinetics) and 14 days (GV, SFO kinetics). DT_{90} values were 0.4 (SW) and 47 days (GV).
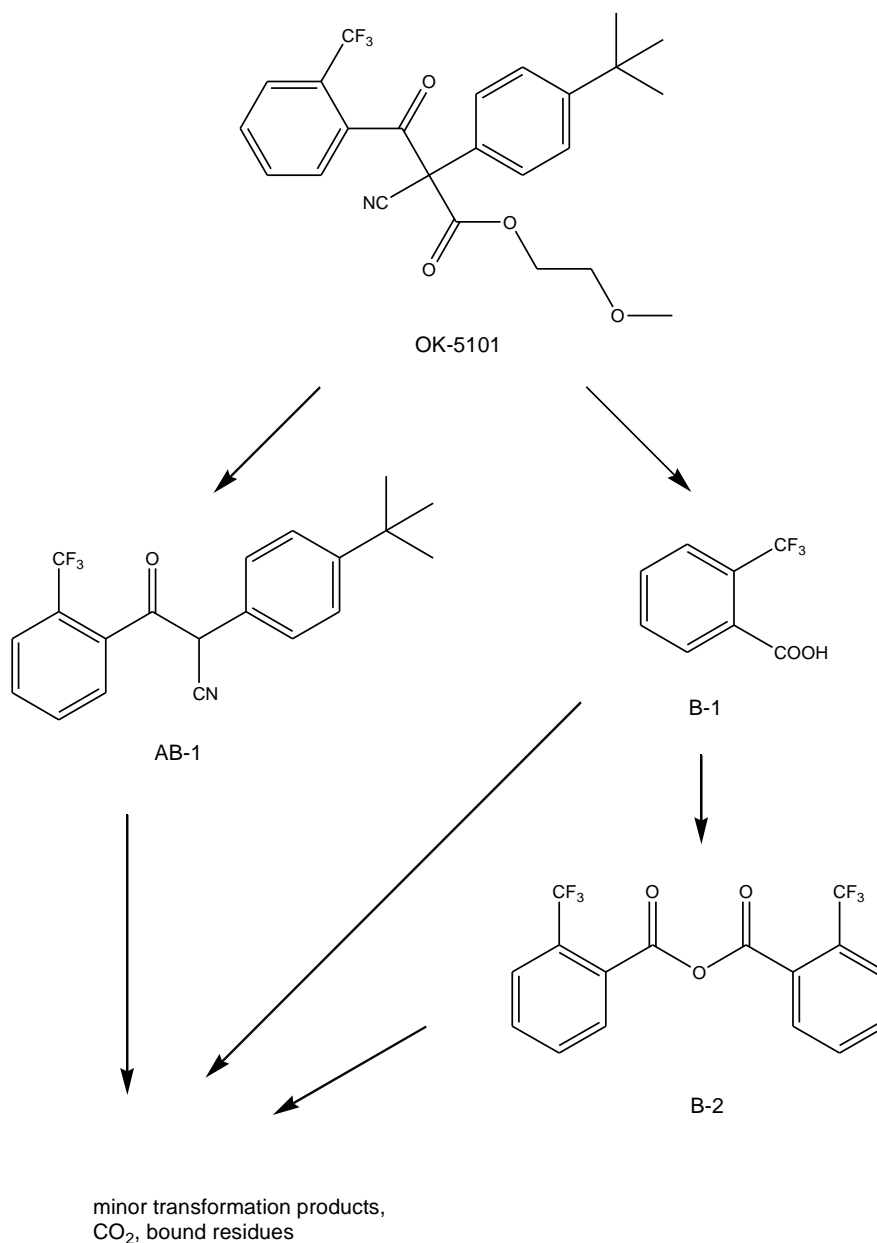
Figure 5 Degradation pathway of cyflumetofen (indicated as OK-5101) in water-sediment (B-label)

5.1.3   Summary and discussion of degradation

The hydrolysis data show that cyflumetofen hydrolyses in the pH range 4-9 with first order DT_{50}s of 7.7 days, 6.0 days, 9.8 hours and 10.3 minutes at pH 4, 5, 7 and 9, respectivley. The major hydrolysis products (>10% of applied radioactivity) at environmentally relevant pH (5-7) are A-1 (14.44% AR), A-2 (44.12% AR), A-18 (36.22% AR), AB-1 (44.51% AR) and B-1 (53.17% AR). Major metabolites were stable (B-1), declined after having reached its maximum concentration (A-1, A-18 and AB-1) or reached its maximum concentration at the end of the test period (A-2). Minor hydrolysis products consisted of unknown fractions all ≤7.76% AR, with the exception of one hydrolysis product at day 14: 11.39% AR (pH 5, B-label). When taking the average of the A- and B-label incubations at pH 5, also this fraction was <10% AR at all time points.

The aquatic photolysis data show that cyflumetofen is susceptible to aquatic photolysis in aqueous buffer solution (pH 5). Cyflumetofen degraded with a half-life of 1.28 hr under irradiated (20 W/m^2, 290-400 nm) conditions and 134.47 hr under dark conditions. In irradiated natural water, the DT_{50} was 1.07 hours. Aquatic photolysis products (in buffer pH 5) exceeding 10% of applied radioactivity were B-1 (11.88% AR), AB-7 (10.82% AR) and AB-15 (54.67% AR). B-1 was also a significant degradation product under dark conditions (max 13.27 % AR). In natural water (dark and irradiated), AB-1 was also observed in significant amounts (max 43.77% AR under dark and 12.45% AR under irradiated conditions). Minor metabolites detected at pH 5 and in natural water were A-1, A-2, A-12, A-14, A-18, AB-1 and AB-6, all ≤ 6.22% AR (pH 5) and ≤ 9.67 (natural water). In summary, the only specific photolytic degradation products of cyflumetofen are AB-15 and AB-7.

The estimated half-life of cyflumetofen in air is 8.2 hours (Atkinson calculation).

The water/sediment studies show that cyflumetofen partitions from the water phase to the sediment and exhibited very low to moderate persistence in the whole system (DT_{50} values range from 0.08 to 14 days). Mineralisation to CO_2 was a minor process in (2-3%), except in the A-label study where 20% mineralisation was observed in the GV test system. Bound residues accounted for maximum 13 to 33% AR. Several relevant degradation products (> 10% of applied radioactivity) were formed in both compartments (AB-11, water max 10.0% AR and sediment max 10.1%; B-1, water max 65% AR and sediment max 21.5% AR) or in the water compartment only (A-2 max 18.4% AR; Met-1 max 10.7% AR; Met-8 max 19.5% AR) or in the sediment compartment only (AB-1 max 14.6%; Met-4 max 10.7%). Metabolite B-2 was formed in the water/sediment study with B-radiolabelled cyflumetofen with a maximum level of 28% AR in the sediment and 15.4% AR in the water compartment. For Met-1 a structure was proposed. Met-4 and 8 remained unidentified (but as they are not observed in the B-label study, they contain only the A-label ring). Where possible, DT_{50} system values were calculted for the metabolites: A-2: 11.74 days, AB-1: 18.29 days; Met-8: 3.09 days; B-1: 320 days; B-2: 0.77 days / 40 days (two systems).

In Section 4.1.2.9 of Annex I of CLP it is stated that "rapid degradation" can be demonstrated by ready biodegradability or other evidence of rapid degradation in the environment (≥ 70% abiotic or biotic degradation in the environment in 28 days). Further, it is stated that primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Data on screening for ready biodegradability are not available.

Based on the results from the simulation tests (water-sediment studies), cyflumetofen appears to be susceptible for primary degradation   (DT_{50}s < 16 days) and not ultimate mineralisation (CO_2

production). For classification and labelling purposes, cyflumetofen is considered not readily degradable. An assessment on the potential for rapid degradability is conducted in Setion 5.5.

5.2   Environmental distribution

5.2.1   Adsorption/Desorption

Adsorption of cyflumetofen was determined by the HPLC method (OECD 121). Due to the instability of cyflumetofen in $CaCl_2$ solutions, it was not possible to determine a Koc for cyflumetofen by the batch adsorption method (OECD 106). The Koc for cyflumetofen was 131826 L/kg, indicating that cyflumetofen will be strongly adsorbed onto soil.

5.2.2   Volatilisation

Based on a low vapour pressure ($<5.9 \times 10^{-6}$ Pa at 25°C) and a medium calculated Henry's law constant of $< 9.4 \times 10^{-2}$ $Pa.m^3.mol^{-1}$ it is concluded that cyflumetofen has a low potential for volatilization. The estimated atmospheric half-life is shorter than 2 days and therefore long-range transport through the atmosphere is not expected.

5.2.3   Distribution modelling

Information not applicable for classification and labelling.

5.3   Aquatic Bioaccumulation

Table 145 Summary of relevant information on aquatic bioaccumulation

Method	Results [L/kg]	Remarks	Reference
Bioconcentration factor (fish) (OECD 305; EC C.13)	170 (for total radioactivity) < 100 (for cyflumetofen)	cyflumetofen was not detected in any fish sample[a]	Bouwman, 2007 (IIA 8.2.6.1/01) [b]

[a] LOD = 0.013 µg/g and 0.11 µg/g for low and high exposure; see Section 5.3.1.2 for further details
[b] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B.9; September 2011.

5.3.1   Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Cyflumetofen

As measured bioaccumulation data are available (see Section 5.3.1.2), the BCF for cyflumetofen was not determined by estimation.

Relevant metabolites

Seven major metabolites were identified to be relevant for surface water: A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8.

For metabolites AB-15 and A-2 a qualitative assessment was conducted considering the available fish bioaccumulation study on cyflumetofen. The molecular structure of AB-15 closely resembles that of parent cyflumetofen (see Annex 8.2 for structural formulars), which is also indicated by the estimated logPow values, which are about similar[2]. Therefore, the uptake of AB-15 in fish is likely to be similar to that of cyflumetofen, and the BCF is taken to be the value determined for total radioactivity in the study with parent cyflumetofen (170). As A-2 is structurally less related to cyflumetofen, for this metabolite the worst-case estimated BCF value is taken (178).

The KOWwin/ACD-Labs-LogP estimated logPow <3 (2.49/2.87; see Annex 8.2) for metabolite B-1 indicates a low potential for bioaccumulation.

The KOWwin/ACD-Labs-LogP estimated logPow>3 for metabolite B-2 (4.26/4.960, AB-7 (4.91/5.00) and AB-11 (5.87/7.32), see Annex 8.2, indicate a potential for bioaccumulation. Metabolite B-2 was only found in the water-sediment study (Noorloos and de Mol, 2007). In the water phase, its maximum level (15.4% of AR) was reached within 0.7 days, after which it was no longer detected (from day 2 onwards). Therefore, prolonged exposure to this metabolite is not expected and bioconcentration is not considered to be of concern for this metabolite. AB-7 was not considered to be ecotoxicologically relevant as it was short lived (only detected at a level >10% after 4 hours (aqueous photolysis; Ohyama K. (2004d))) and hence bioconcentration is not considered of concern for this metabolite. AB-11 was only detected at major (>10%) levels after 0.7 days (10.0% AR, water sediment study, Noorloos and Brands, 2008), after which measured levels decreased to below 5% (4.7, 1.5, 1.0 and 0% AR after 2, 5, 12 and 29 days). Hence the DT_{50} of this metabolite is clearly < 2 days. Therefore, prolonged exposure to AB-11 is not expected, and bioconcentration is not considered to be of concern for this metabolite.

Met-1 and Met-8 are unidentified metabolites and no further assessment was made for these two compounds.

5.3.1.2 Measured bioaccumulation data

Cyflumetofen

Reference	:	Bouwman, L.M. (2007)	GLP statement	:	Yes
Type of study	:	Bioconcentration test in carp	Guideline	:	OECD 305; EC C.13
Year of execution	:	2007 (with report amendment of 2009)	Deviations	:	None
Test substance	:	Cyflumetofen	Acceptability	:	Acceptable
Exposure regime	:	21 flow-through exposure, followed by 32 days depuration	BCF	:	<100 for cyflumetofen; 170 for cyflumetofen equivalents

Materials and Methods

Cyflumetofen; Batch No. 01H1; Purity 98.4% and B-[ring-U-^{14}C]Cyflumetofen, Batch CFQ14971 Batch 1, Purity 99.9%, radiochemical purity 99.0-99.5% were used in the study. Carp, *Cyprinus carpio*, (body weight 1.95 ± 0.74 g, body length 4.2 ± 0.5 cm) were exposed to B-[ring-U-^{14}C]Cyflumetofen for 21 days in a flow-through system, followed by 32 days of depuration in clean water. Nominal concentrations of 1.0 and 10 µg/L, plus solvent control (acetone, 0.1 mL/L) were each tested in one replicate aquarium containing 48 fish at test initiation (22 fish were used for the solvent-control). Fish biomass at study start was 0.24 g/L/24 hours.

---

[2] Calculated values (KOWwin/ACD-Labs-LogP) are 4.69/6.18 and 5.05/5.87 for cyflumetofen and AB-15, respectively

Water samples (100-300 mL) were removed from treated and control aquaria on day 0, 1, 3, 7, 10 and 21 of uptake. Four and two fish, respectively, were sampled from treated and control aquaria on day 1, 3, 7, 10 and 21 of uptake and 2, 7, 14 and 29 of depuration.

Water samples were extracted with dichloromethane, concentrated and re-constituted in acetonitrile. Fish samples were extracted with buffer pH 4/acetonitrile (1:5). The buffer/acetonitrile extract was not further partitioned or concentrated. The lipid content was determined by evaporation of the chloroform extracts and weighing of the dried residue.

Radioactivity in all extracts and fractions was quantified by LSC by combustion/LSC. Radioactivity in organic extracts of water and fish samples was identified by TLC. Compound identification was by co-chromatography with a reference standard (cyflumetofen). The aqueous fractions of fish and water were not analysed by TLC.

Validation of the extraction method of the water samples was provided by analysis of samples of control water fortified with B-[ring-U-^{14}C]Cyflumetofen (1 and 10 µg/L): recoveries of total radioactivity ranged from 99 to 102%. The concentrated extracts contained 92-96% cyflumetofen based on TLC analysis. Validation of the extraction method of the fish samples was provided by concurrent analysis of samples of control fish fortified with B-[ring-U-^{14}C]Cyflumetofen (0.1 and 0.1 µg/g): it was reported that cyflumetofen was extracted efficiently (recoveries of radioactivity 97-104%). However, cyflumetofen only represented 45-50% of the radioactivity. It was concluded that cyflumetofen degraded immediately after spiking on fish.

Temperature was measured continuously, and dissolved oxygen and pH were measured three times a week. Total hardness and conductivity of the test medium were measured at day 0, 21 and 53. Dissolved organic carbon in the control was measured once weekly.

Results and discussion

Environmental conditions

Water quality parameters were: 16 h light, 8 h dark; 20-22°C; dissolved oxygen >60% of saturation; pH 7.0-8.1; TOC 41-53 mg/L during exposure and ≤2 mg/L during depuration (hence TOC mainly attributable to solvent); hardness 143-179 mg CaCO$_3$/L.

Chemical analysis

The radiochemical purity of the stock solutions in acetone were 100.0% (10 mg/L) and 99.4% (100 mg/L).

It was reported that cyflumetofen represented 44-73% of the radioactivity in extracts of water. Based on TLC analysis the mean concentrations of parental cyflumetofen were 0.43 and 5.2 µg a.s./L, respectively, at low and high exposure. The total recovery of radioactivity in fish samples was 76-96% TRR (based on LSC), of which 52-69% TRR was extractable with buffer/acetonitrile. Two unidentified degradates were detected at levels up to 59.5% TRR (Met A) and 15.5% TRR (Met C). These degradates were also detected in the test medium (Met A: max. 5.1% TRR; Met C: max. 25.4% TRR). Cyflumetofen was not detected in any fish sample. However, concurrent recovery samples showed that cyflumetofen in carp partly degraded during sample processing or otherwise could not be detected as such (only 35-67% of applied cyflumetofen was recovered on TLC). Following from this, approximately 50% of the cyflumetofen present in fish extracts was expected to be detected on TLC. Based on the LOD (0.013 µg/g and 0.11 µg/g for low and high

exposure, respectively), it can be concluded that the concentrations of cyflumetofen in fish extracts were lower than 0.026 µg/g and 0.23 µg/g (low and high exposure, respectively).

The radioactivity concentration in whole fish increased from day 1 to day 3, when a steady-state situation was reached. The reported steady-state bioconcentration factor (BCF) values were calculated by dividing the mean ^{14}C concentration in fish during day 3-21 by the mean ^{14}C concentration in water during day 3-21. In addition, BCF values were calculated as k_1/k_2, where k_1 and k_2 are the uptake and depuration rate constant, estimated according to the methods outlined in Annex 6 of OECD 305 (1996). The results for whole fish of all estimation methods are shown in Table 146. Kinetic BCF values were somewhat higher than BCF values based on measured radioactivity concentrations. This is probably caused by the fact that depuration occurred in a biphasic manner, while the calculation of k2 (and hence k1) was based on the assumption of a simple first order model. Considering the recovery of parent cyflumetofen in fish samples (<0.026 and <0.23 µg/g at low and high exposure, respectively, rounded up to <0.03 and <0.3 µg/g), the BCF for parent cyflumetofen was <70 and <58 L/kg for low and high exposure, respectively.

The CT_{50} value for radioactivity in whole fish was 2.2-2.5 days. The CT_{90} value was 7.4-8.3 days.

The mean lipid content in fish was 1-6% at day 8 (fish from stock culture) and 4-5% at day 21 (fish taken from the study). The value of 1% measured on day 8 was reported to be an anomaly, as previous lipid extractions during bioconcentration studies showed a mean value of 5%.

Table 146 Bioconcentration factors (L/kg wwt) for radioactivity and cyflumetofen in whole fish exposed to B-[ring-U-^{14}C]Cyflumetofen at nominal exposure concentration 1.0 or 10 µg a.s./L

Parameter	1.0 µg/L nominal		10 µg/L nominal	
	14C	Cyflumetofen	14C	Cyflumetofen
steady state BCF[(A)]	170	na	146	na
kinetic BCF	202	na	171	na
k_{uptake} (day^{-1})	56.0	na	53.5	na
$k_{depuration}$ (day^{-1})	0.278	na	0.313	na
CT_{50} (day)	2.5	na	2.2	na
lipid BCF[(A)(B)]	34	na	29	na

(A) Based on day 3-21 average concentration in fish and water.
(B) Normalised to 1% fat; based on day 8, and 21 average lipid content of fish.
na = not applicable (cyflumetofen was not detected in fish samples)

Clinical symptoms

No clinical effects were observed on the fish during the test period.

Conclusions

BCF values for total radioactivity in whole fish were 170 and 146 L/kg wwt at 1.0 and 10 µg a.s./L respectively (lipid BCF normalised to 1% fat 34 and 29 L/kg wwt); CT_{50} for radioactivity in whole fish was 2.2-2.5 days. CT_{90} was 7.4-8.3 days. Since cyflumetofen was not detected in any fish sample, the BCF for cyflumetofen was <100 at both exposure levels.

Relevant metabolites.

No measured bioaccumulation data are available for the seven major metabolites identified to be relevant for surface water (A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8).

5.3.2   Summary and discussion of aquatic bioaccumulation

A fish bioconcentration study is available for parent cyflumetofen (BCF < 100).

Following the CLP Regulation, a BCF in fish of ≥500 is indicative of the potential to bioconcentrate for classification purposes. Cyflumetofen is concluded to have a low potential to bioconcentrate because it does not meet the criterion.

5.4   Aquatic toxicity

Table 147 lists the results from the relevant aquatic acute toxicity studies that were performed with cyflumetofen and the major aquatic degradation products AB-11, B-1 and B-2. The key acute and chronic studies carried out with cyflumetofen at each trophic level are described in more detail below. Chemical structures of the metabolites are shown in Annex 8.2.

Table 147 Summary of relevant information on aquatic toxicity (including sediment)

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [a] [mg/L]	Remarks
Acute toxicity to fish							
ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Oncorhynchys mykiss* (rainbow trout)	96h (flow-through)	Survival, LC_{50}	>0.63 (mm)	Migchielsen, 2003a, (IIA 8.2.1.1/01) [d]
ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Cyprinus carpio* (carp)	96h (flow-through)	Survival, LC_{50}	>0.54 (mm)	Migchielsen, 2003b (IIA 8.2.1.1/02) [d]
Acute toxicity to invertebrates							
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.0%	*Daphnia magna*	48h (flow-through)	Immobility, EC50	>0.063 (mm)	Bouwman, 2003a (IIA 8.3.1.1/01) [d]
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.4%	*Daphnia magna*	48 h (flow-through)	Immobility, EC50	0.70 (im) [f]	Migchielsen 2009b (IIA 8.3.1.1/02) [d]
ISO 6341; EEC C.2; OECD 202	AB-11	99.6%	*Daphnia magna*	48h (static)	Immobility, EC50	>0.5 (nom) or >0.476 (mm)	Migchielsen, 2009a (IIA 8.3.1.1/03) [d]
ISO 6341; EEC C.2; OECD 202	B-1	99.99%	*Daphnia magna*	48h (static)	Immobility, EC50	>180 (nom)	Migchielsen, 2008b (IIA 8.3.1.1/04) [d]
ISO 6341; EEC C.2; OECD 202	B-2	99.9%	*Daphnia magna*	48h (static)	Immobility, EC50	>0.039 (im) or >0.0062 (mm)	Bouwman, (2009a) IIA 8.3.1.1/05) [d]
Acute toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate, EC_{50}	>0.30 (im) or >0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, EC_{50}	>0.5 (nom) or >0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneriella subcapitata*	96h (static)	Yield/growth rate, EC_{50}	>100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, EC_{50}	>0.073 (im) or >0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]

Chronic toxicity to fish							
OECD 212	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	8d (flow-through)	Survival/hatching, NOEC	≥ 0.145 (mm)	Migchielsen, 2008a (IIA 8.2.4/01) [d]
OECD 215	cyflumetofen	98.4%	*Cyprinus carpio* (carp)	28d (flow-through)	Survival/growth, NOEC	0.072 (mm)	Migchielsen, 2007a (IIA 8.2.3/01) [d]
OECD 210	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	31d (flow-through)	Hatching/larval survival/growth, NOEC	0.054 (mm)	Migchielsen, 2010 [e]
Chronic toxicity to invertebrates							
OECD 211; ISO International Standard 10706:2000 (2000-03-30)	cyflumetofen	98.4%	*Daphnia magna*	21d (flow-through)	Mortality, NOEC	0.065 (mm) [b]	Migchielsen, 2007b (IIA 8.3.2.1/01) [d]
Chronic toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate, NOEC [c]	0.30 (im) or 0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, NOEC [c]	0.5 (nom) or 0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneriella subcapitata*	96h (static)	Yield/growth rate, NOEC [c]	100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, NOEC [c]	0.073 (im) or 0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]
Sediment dwelling organisms							
OECD 219	cyflumetofen	98.4%	*Chironomus riparius*	28d (static)	Emergence/development (water spiked), NOEC	≥ 0.064 (im)	Desmares-Koopmans, 2009a (IIA 8.5.2/01) [d]
OECD 218	AB-1	99.8%	*Chironomus riparius*	28d (static)	Emergence/development (sediment spiked), NOEC	59.6 mg/kg (im)	Desmares-Koopmans, 2009b (IIA 8.5.2/02) [d]

[a] (mm): mean measured concentrations; (nom): nominal; (im): initially measured

[b] This study is less reliable due to high mortality in the control. However, the experts in the Pesticides Peer Review Expert Meeting considered that a new chronic study with daphnids for cyflometofen is not required based on the following arguments: in the study, no effects were seen on reproduction (thus: NOECreproduction ≥151 µg a.s./L); the chronic NOEC for daphnids of 65 µg a.s./L is based on mortality which is a worst case approach; the NOEC of 65 µg a.s./L is comparable to the acute NOEC for daphnids.

[c] The NOEC was taken from the original study report as this endpoint was not reported in the EFSA evaluation and the DAR.

[d] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B.9; September 2011.

[e] This study (Migchielsen, 2010: Fish early-life stage toxicity test with OK-5101 (flow-through), NOTOX B.V., 's-Hertogenbosch, The Netherlands. Unpublished report No. 487052) was submitted as supplement to the DAR. Note: "OK-5101" is used as code for "cyflumetofen".

[f] This study was considered acceptable as supplementary information, as 50% of the daphnids were immobilised at the limit concentration. Therefore the endpoint was not considered reliable for risk assessment during the EU review and was not taken to the LoEP, but the test was concluded to indicate that at a test concentration of at least a factor of 10 higher than that in the first test, the effects seen were still only ca. 50% and were probably at least partly caused by mechanical damage of the undissolved material.

Metabolites A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8 were identified as being relevant for the aquatic compartment (B-3 was formed in soil and may enter the surface water via drainage/run-off).

Toxicity data for water column living organisms are only available for three of the relevant metabolites (AB-11, B-1 and B-2). These data cover acute toxicity for daphnids and algae. For the remaining metabolites, Ecosar (EPA Epi Suite software) predictions were made. ECOSAR uses a number of chemical class-specific log Kow-based QSARs in order to predict the toxicity of chemicals to aquatic organisms (fish, daphnids, green algae). Validation of the predictions by means of QMRF (QSAR Model Reporting Format) and QPRF (QSAR Prediction Reporting Format) documentation is considered to be out of the scope of this CLH proposal as the resulting predictions are not actually used in the classification and labelling of cyflumetofen.

At the time the predictions were made (2007), the validity of the predictions was based on comparison with experimental data where available and expected (lower) toxicity based on the presence or absence of the active moiety of the parent molecule. This approach was accepted during the EU review of cyflumetofen for active substance approval (see below). Since 2007, Ecosar has been updated several times, and the training data sets are no longer the same sets as in 2007. Therefore, it is not possible to assess the performance of training dataset used for the predictions for cyflumetofen in a retrospective assessment, as it is not known which substances were included at the time the estimations were made. However, regarding applicability domain the following data are still available: Ecosar generated warnings only for for B-1 and AB-15 (i.e. "chemical may not be soluble enough to measure this predicted effect"), and the cut-off values for logKow were always indicated to be 5.0 for *Daphnia* and 6.4 for algae. This is sufficient for most predictions.

The table below contains predicted and measured toxicity data for the relevant water metabolites and cyflumetofen. As comparison between predicted and measured toxicity was part of the evaluation of the predictions, the data for cyflumetofen have also been included in the table. The evaluation of the Ecosar predictions included in the DAR stated:

AB-11
AB-11 was predicted to be more toxic than parent cyflumetofen. In laboratory testing, cyflumetofen was not toxic at the highest tested concentration (0.063-0.30 mg/L), which was limited by its low water solubility (i.e. 0.028 mg/L). AB-11 was also not toxic at the highest tested concentration (0.5 mg/L), which was also limited by its water solubility. As both cyflumetofen and AB-11 were not

toxic at concentrations as high as their maximum solubility level in test medium and orders of magnitude over their PECsw values, it can be concluded that AB-11 is not more toxic than parent cyflumetofen.

B-1
B-1 was predicted to be less toxic than parent cyflumetofen. In laboratory testing, cyflumetofen was not toxic at the highest tested concentration (0.063-0.30 mg/L), which was limited by its low water solubility (i.e. 0.028 mg/L). B-1 was not toxic at the highest tested concentration, which was orders of magnitude higher than that of cyflumetofen (100-180 mg/L). Therefore, it can be concluded that B-1 was not more toxic than parent cyflumetofen.

B-2
B-2 was predicted to be less toxic than parent cyflumetofen. In laboratory testing, cyflumetofen was not toxic at the highest tested concentration (0.063-0.30 mg/L), which was limited by its low water solubility (i.e. 0.028 mg/L). B-2 was also not toxic at the highest tested concentration (im 0.039-0.073 mg/L), which was also limited by its water solubility. As both cyflumetofen and B-2 were not toxic at concentrations as high as their maximum solubility level in test medium, it can be concluded that B-2 is not more toxic than parent cyflumetofen.

Conclusion on predictions of toxicity by Ecosar
Following from the above described results, it is concluded that Ecosar predictions were sometimes conservative (i.e. overestimated toxicity for AB-11), but did not underestimate the toxicity of any of the tested metabolites. Consequently, the predicted lower toxicity for AB-15, A-2 and B-3 was considered to be sustainable and useful for qualitative risk assessment. In the risk assessment, it was assumed that AB-15, A-2 and B-3 were not more toxic than parent cyflumetofen. The predicted toxicity of metabolite 1 is close to that of AB-11. Metabolite 1 and AB-11 are structurally related. However, the tentative structure of metabolite 1 does not contain the active moiety of cyflumetofen, while AB-11 does. It is therefore expected that the toxicity of metabolite 1 is an overestimation. AB-11 was concluded to be not more toxic than parent cyflumetofen. Consequently, it is concluded that metabolite 1 is not more toxic than parent cyflumetofen. Furthermore, the ecological relevance of metabolite 1 is unclear, as this metabolite was only found at a level slightly >10% AR once (10.7% AR after 59 days; ≤1.7% AR on days 8, 15, 30 and 98). Therefore, the ecological risk of metabolite 1 is low.

Met-8 remained unidentified and hence, toxicity could not be predicted or tested. Met-8 was found in the water-sediment study with A-ring labelled cyflumetofen, and not in the study with B-ring labelled cyflumetofen. This indicates that Met-8 is not an AB-metabolite, but rather an A-metabolite. As A-metabolites have lost the active moiety of cyflumetofen, Met-8 is expected to be not more toxic than parent cyflumetofen.

In conclusion, none of the relevant water metabolites of cyflumetofen was expected to be more toxic than parent cyflumetofen.

Table 148 Acute toxicity of metabolites of cyflumetofen to aquatic organisms: predicted and experimentally determined endpoints

Metabolite [a]	Predicted [b] logKow	Predicted [b] water solubility [mg/L]	Daphnia magna L(E)C$_{50}$ [mg/L]		Algae L(E)C$_{50}$ [mg/L]	
			Predicted [b]	Experimental [c]	Predicted [b]	Experimental [c]
cyflumetofen	4.69	3.598	1.267	>0.063	0.217	>0.0396
AB-11	5.87	0.2165	0.103	>0.476	0.051	>0.157
AB-15	5.05	1.541	5.96	-	4.441	-
A-2	3.47	24.39	6.324	-	4.303	-
B-1	2.49	267.4	541	>180	348	>100
B-2	4.26	7.976	2.526	>0.039	1.798	>0.0101
B-3	0.97	9449	1300	-	766	-
Metabolite 1	6.21	0.08862	0.046	-	0.031	-

[a] AB-11 and AB-15 contain the active site of cyflumetofen; A-2, B-1, B-2, B-3 and Met-1 (tentative structure) do not contain the active site of cyflumetofen

[b] Using EPA Epi Suite software (Ecosar); predicted water solubilities are not reported in the DAR

[c] Results from aqua toxicity testing in the laboratory. Endpoints were all above the highest tested concentration, which was in many cases limited by the water solubility of the test substance. Experimental L(E)C$_{50}$ values are based on mean measured, analytically confirmed nominal (B-1) or initial (B-2) concentration.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

The acute toxicity of cyflumetofen (purity 98.0%) for both *Oncorhynchys mykiss* (rainbow trout) and *Cyprinus carpio* (carp) was tested in a 96h flow-through study in accordance with ISO, EC and OECD guidelines.

Juvenile Rainbow trout, (body weight 2.95 ± 0.41 g, body length 5.6 ± 0.53 cm) were exposed to a nominal concentration of 1.0 mg of the test substance/L, using acetone as solvent carrier. The pH varied between 7.8 and 8.0, the oxygen concentration was 9.4-9.8 mg/L and temperature was maintained at 15.0-15.4°C during the test. The mean measured concentration was 0.63 mg cyflumetofen /L, which exceeded the water solubility of cyflumetofen (reported to be 28 μg/L). During the test, small amounts of precipitate and a thin floating layer were observed in the solution with cyflumetofen, but this was reported not to have affected the fish. Symptoms of toxicity were noted in the fish exposed to cyflumetofen within 24 hours of exposure and included slower swimming and discolouration. The LC$_{50}$ was >0.63 mg/L.

Table 149 Acute toxicity of cyflumetofen to Rainbow trout under flow-through conditions

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]	Mortality after 96 h [%]
Solvent-control	Solvent-control	0
1.0	0.63	0

Juvenile carp (body weight 0.46 ± 0.05 g, body length 2.6 ± 0.21 cm) were exposed to a nominal concentration of 1.0 mg of the test substance/L using acetone as solvent carrier. The pH varied between 7.8 and 8.0, the oxygen concentration was 9.0-9.6 mg/L and temperature was maintained at 20.9-21.5°C during the test. The mean measured concentration was 0.54 mg cyflumetofen/L, which exceeded the water solubility of cyflumetofen. During the test, small amounts of precipitate and a thin floating layer were observed in the solution with cyflumetofen, but this was reported not to have affected the fish. No mortality or symptoms of toxicity were noted in the fish exposed to cyflumetofen. The LC_{50} was >0.54 mg/L.

Table 150 Acute toxicity of cyflumetofen to carp under flow-through conditions

Nominal concentration [mg a.s./L]	Mean measured concentration [mg a.s./L]	Mortality after 96 h [%]
Solvent-control	Solvent-control	0
1.0	0.54	0

5.4.1.2 Long-term toxicity to fish

Fathead minnow, *Pimephales promelas*, (freshly fertilised eggs) were exposed to cyflumetofen (purity 98.4%) at target concentrations of 10, 46 and 220 µg/L in a flow-through test design for 8 days in accordance with OECD test guideline 212. A mixture of DMF (dimethylformamide) and Cremophor (1:1) was used as solvent carrier. The pH varied between 7.6 and 7.9, the oxygen concentration was 8.3-8.7 mg/L and temperature was maintained at 22-26°C during the test. Mean measured concentrations in the test were 5.9, 27 and 145 µg/L. During the test, no test substance precipitation was observed. Egg survival during the first 24 hours was 93% in the solvent-control and ≥87% in all test concentrations. Hatching success was not affected at any concentration. Larval survival was reduced at 145 µg/L, but this slight reduction was not statistically significant. The NOEC was 145 µg/L.

Table 151 Toxicity of cyflumetofen to egg and sac-fry stages of fathead minnow under flow-through conditions

Target concentration [µg a.s./L]	Mean measured concentration [µg a.s./L]	Egg survival after 24 hours [%]	Hatching rate [%]	Survival of eggs and larvae after 8 days [%]
Blank-control	Blank-control	93	61	93
Solvent-control	Solvent-control	93	100	80
10	5.9	97	100	90
46	27	100	100	97
220	145	87	100	73

Juvenile carp, *Cyprinus carpio*, (body weight 1.2 ± 0.22 g, body length 3.5 ± 0.24 cm at the start of the test) were exposed to cyflumetofen (purity 98.4%) at target concentrations of 10, 22, 46, 100 and 220 µg/L in a flow-through test design for 28 days in accordance with OECD test guideline 215. A mixture of DMF (dimethylformamide) and Cremophor (1:1) was used as solvent carrier. The pH varied between 7.1 and 7.8, the oxygen concentration was 5.8-9.0 mg/L and temperature was maintained at 21-23°C during the test. Mean measured concentrations in the test were 7.2, 16, 34, 72 and 179 µg/L at target concentrations of 10, 22, 46, 100 and 220 µg/L, respectively. During the test, no test substance precipitation was observed. No mortality or symptoms of toxicity were noted in the fish exposed to cyflumetofen, except for one fish exposed to 179 µg/L that died on day 22.

Increase in body weight, growth rate and body length were significantly affected at 179 µg/L. The NOEC was 72 µg cyflumetofen/L.

Table 152 Chronic toxicity of cyflumetofen to carp under flow-through conditions

Target Concentration [µg a.s./L]	Mean measured concentration [µg a.s./L]	Mortality after 28 days [%]	Increase in mean fish weight after 28 days [%]	Growth rate between days 0 and 28 [g/day]	Mean fish length after 28 days [cm]
Blank-control	Blank-control	0	+126	2.662	2.60
Solvent-control	Solvent-control	0	+128	2.951	2.81
10	7.2	0	+114	2.623	2.59
22	16	0	+119	2.759	2.63
46	34	0	+125	2.820	2.68
100	72	0	+118	2.542	2.53
220	179	8	+53*	1.411*	1.87*

Fathead minnow (*Pimephales promelas*) eggs (freshly fertilised) were exposed to cyflumetofen (purity 98.4%) at target concentrations of 15, 30 and 60 µg/L in a flow-through test design for 31 days in accordance with OECD test guideline 210. Acetone was used as a solvent carrier. The pH varied between 7.3 and 8.1, the oxygen concentration was 6.9-9.2 mg/L and temperature was maintained at 24.6-27.2ºC during the test. Mean measured concentrations were 11, 34 and 54 µg/L. No test substance precipitation was observed during the test. Egg survival during the first 24 hours was 90% in the solvent-control and ≥80% in all test concentrations. Hatching success, larval survival, body length and body weight were not affected at any tested concentration. No treatment-related sub-lethal effects were observed in any test concentration. The NOEC was 54 µg cyflumetofen/L, which exceeded water solubility.

Table 153 Chronic toxicity of Cyflumetofen to fathead minnow (ELS) under flow-through conditions

Target Concentration [µg a.s./L]	Mean measured concentration [µg a.s./L]	Egg survival after 24 hours [%]	Hatching rate [% of survivors at 24 hours]	Survival of eggs and larvae after 31 days [% of hatched]	Body length [mm]	Body weight [mg]
Blank-control	Blank-control	90	93	82	16.3	78.0
Solvent-control	Solvent-control	90	94	73	17.5	90.7
15	11	85	90	61	17.8	98.0
30	34	87	88	46	17.0	82.4
60	54	85	96	56	17.1	77.0

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Juvenile *Daphnia magna* (<24 hours old) were exposed to cyflumetofen (purity 98.0%) at a nominal concentration of 0.1 mg/L in a flow-through test design for 48 hours in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of cyflumetofen (28 µg/L); acetone was used as solvent carrier. The pH varied between 7.5 and 7.8, the oxygen concentration was 9.4-9.8 mg/L and temperature was maintained at 20.2-20.9°C during the test. The mean measured concentration was 0.063 mg/L, which exceeded the water solubility of

cyflumetofen. During the test, a floating layer was observed on the solution with cyflumetofen, but this was reported not to have affected the daphnids. No symptoms of toxicity were found throughout the test period. The 48-h EC_{50} was >63 µg/L.

Table 154 Acute toxicity of Cyflumetofen to *Daphnia magna* under flow-through conditions

Concentration [mg a.s./L]		[%] Immobilisation after	
Nominal	**Measured mean**	**24 h**	**48 h**
Solvent-control	0	0	0
0.100	0.063	0	0

The above described study with cyflumetofen was repeated applying an increased test dose which was however hampered by the low solubility of cyflumetofen (Migchielsen 2009c; 8.3.1.1/02). In this second test, 50% of the daphnids were immobilised at the limit dose of 0.70 mg/L. According to the guideline no effects should occur at the tested dose in a limit test. RMS considered an EC_{50} of 0.70 mg/L therefore not reliable for use in the risk assessment. However, the test does indicate that at a test dose of at least about a factor of 10 higher than that in the first test, the effects seen are still only ca. 50% and are probably at least partly caused by mechanical damage of the undissolved material.

Table 155 Acute toxicity of cyflumetofen to *Daphnia magna* under flow-through conditions

Concentration [mg a.s./L]		[%] Immobilisation after	
Nominal	**Measured initial**	**24 h**	**48 h**
Solvent-control	0	0	0
1.0	0.70	0	50

Metabolites

Juvenile *Daphnia magna* (<24 hours old) were exposed to AB-11 (purity 99.6%) at a nominal concentration of 500 µg/L in a static test design for 48 hours in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of AB-11 (predicted water solubility of 216.5 µg/L, see Table 147); acetone was used as solvent carrier. The pH varied between 7.7 and 7.9, the oxygen concentration was 8.8-9.0 mg/L and temperature was maintained at 19.5-21.4°C during the test. The mean measured concentration in the test was 476 µg/L. After 24 and 48 hours of exposure, respectively, 65% and 40% of the daphnia exposed to AB-11 was trapped at the surface, and 0 and 45% of the daphnia were immobilized. The main cause for the observed immobility was expected to be the fact that testing was performed above the actual limit of solubility. It was clearly observed that daphnia were exposed to a finely dispersed but homogeneous oversaturated test solution. Hence, the visible small undissolved particles were expected to have interfered with the swimming capacity of the daphnia despite the fact that daphnia were not covered by undissolved material (microscopic observation). This further resulted in a relatively high number of organisms that were trapped at the surface. Although according to the guideline no effects should occur at the tested dose in a limit test, the conclusion remains that the EC_{50} for daphnia exposed to AB-11 exceeds its limit of solubility in test medium and hence the 48-h EC_{50} values were >500 µg/L (nominal) and >476 µg/L (mean measured).

Table 156 Acute toxicity of AB-11 to *Daphnia magna* under static conditions

Concentration [µg/L]		[%]Immobilisation after	
Nominal	Measured mean	24 h	48 h
Solvent-control	0	0	0
500	476	0	45

[a] Physical effects; testing was performed above the actual limit of solubility.

Juvenile *Daphnia magna* (<24 hours old) were exposed to B-1 (purity 99.99%) at nominal concentrations of 10, 18, 32, 56, 100 and 180 mg/L in a static test design for 48 hours in accordance with ISO, EC and OECD guidelines. The test substance was dissolved in test medium. The pH varied between 6.1 and 8.0, the oxygen concentration was 8.0-9.0 mg/L and temperature was maintained at 19.5-20.0°C during the test. The mean measured concentration at the highest tested concentration was 178 µg/L. No symptoms of toxicity were found throughout the test period. The 48-h EC_{50} value was >180 mg/L.

Table 157 Acute toxicity of B-1 to *Daphnia magna* under static conditions

Concentration [mg/L]		[%]Immobilisation after	
Nominal	Measured mean	24 h	48 h
Blank-control	0	0	0
10	Not measured	0	0
18	Not measured	0	0
32	Not measured	0	0
56	Not measured	0	0
100	Not measured	0	0
180	178	0	5

Juvenile *Daphnia magna* (<24 hours old) were exposed to B-2 (purity 99.9%) at a nominal concentration of 500 µg/L in a static test design for 48 hours in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of B-2; acetone was used as solvent carrier. The pH varied between 7.7 and 8.0, the oxygen concentration was 9.0 mg/L and temperature was maintained at 19.8-21.5°C during the test. The measured initial concentration was 39 µg/L; the mean measured concentration was 6.2 µg/L. No symptoms of toxicity were found throughout the test period. The 48-h EC_{50} value was >39 µg/L (initially measured) or >6.2 µg/L (mean measured).

Table 158 Acute toxicity of B-2 to *Daphnia magna* under static conditions

Concentration [µg/L]			[%] Immobilisation after	
Nominal	Measured initial	Mean measured	24 h	48 h
Control	0	0	0	0
500	39	6.2	0	5

The above described study with B-2 followed a static test design. Following the instability of B-2 in water, several attempts were undertaken to prepare stable solutions of B-2 in test medium that could be used for an acute toxicity study in Daphnia under flow-through conditions (Bouwman, 2013; IIA1 8.3.1/01). One and 2 days after the start of the flow-through system, the measured concentration represented 14-24% and 11-17% of nominal, respectively. Seven days after the start of the system, the concentration of B-2 was below the limit of detection. It was concluded that it is technically impossible to perform an acute toxicity study in Daphnia magna with B-2 under flow-through conditions in which the exposure levels can be maintained at a constant level. In addition to

the above, EPISUITE was run for B-2. EPISUITE (hydrowin v2.00) indicated that the hydrolysis rate is short, with half-lives of 18.240 minutes at pH 7 and 1.824 minutes at pH 8. As a consequence, the conclusion of the study performed by Bouwman (2013, study 10.10/01) is considered appropriate. In case of rapidly hydrolysing substances, it is considered justified to base the toxicity endpoint on the measured initial concentration. Therefore, the study by Bouwman (2013) and the estimation by EPISUITE indicate that it is justified to base the acute endpoint of B-2 to *Daphnia magna* on measured initial concentrations. Thus, the EC_{50} of B-2 to *Daphnia magna* is >0.039 mg/L (Bouwman 2009a, IIA 8.3.1.1/04 in the DAR).

5.4.2.2 Long-term toxicity to aquatic invertebrates

Juvenile *Daphnia magna* (<24 hours old) were exposed to cyflumetofen (purity 98.4%) at target concentrations of 10, 22, 46, 100 and 220 µg/L in a flow-through test design for 21 days in accordance with ISO and OECD guidelines. A mixture of DMF (dimethylformamide) and Cremophor (1:1) was used as solvent carrier. The pH varied between 7.4 and 8.6, the oxygen concentration was 6.2-8.9 mg/L and temperature was maintained at 21-22°C during the test. Mean measured concentrations in the test were 7.5, 12, 32, 65 and 151 µg/L at target concentrations of 10, 22, 46, 100 and 220 µg/L, respectively. Mortality in the test solutions varied between 15% at 7.5 µg/L and 45% at 151 µg/L. Mortality at 151 µg/L was significantly different from that in the solvent-control after 20 days. Parental body length was not affected at any concentration. Appearance of first brood was not delayed at any concentration. No immobile young or unhatched eggs were observed in any group. Reproduction was not affected at any concentration. Under flow-through conditions the 21-day NOEC of cyflumetofen to *Daphnia magna* was 65 µg/L, based on a significant effect on parental survival after 20 days.

The mortality in the blank control of the above study was far above the validity criterion of the guideline of ≤20% (58%). Mortality in the solvent control was 28%, closer to the validity criterion but still too high. The solvent control was used for comparison to the treatment groups. Since the study does not fulfil the validity criterion of the relevant guidelines, the reliability of the study was discussed by the experts in the Pesticides Peer Review Expert Meeting. It was concluded that a new chronic study with daphnids for cyflometofen is not required based on the following arguments: in the study, no effects were seen on reproduction (thus: $NOEC_{reproduction} \geq 151$ µg/L); the chronic NOEC for daphnids of 65 µg/L is based on mortality which is a worst case approach; the NOEC of 65 µg/L is comparable to the acute NOEC for daphnids.

Table 159 Chronic toxicity of cyflumetofen to parental *Daphnia magna* under flow-through conditions

Target Concentration [µg a.s./L]	Mean measured concentration [µg a.s./L]	Mortality after 20 days [%]	Mortality after 21 days [%]	Mean body length after 21 days [mm]
Blank-control	Blank-control	53	58	4.2
Solvent-control	Solvent-control	13	28	4.1
10	7.5	15	15	4.3
22	12	28	28	4.2
46	32	25	33	4.2
100	65	20	25	4.2
220	151	43*	45	4.1

5.4.3 Algae and aquatic plants

Green algae, *Selenastrum capricornutum*, (initial cell density 10000 cells/mL of test medium) were exposed to cyflumetofen (purity 98.0%) at a nominal test concentration of 10 mg/L for 72 hours under static conditions in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of cyflumetofen (i.e. 28 µg/L); acetone was used as solvent carrier. The pH varied between 7.3 and 8.9 and temperature was maintained at 22.7-24.1°C during the test. The initial measured concentration of 0.30 mg/L exceeded the water solubility of cyflumetofen. The mean measured concentration in the test was 0.0396 mg/L. Cyflumetofen did not inhibit cell growth or reduce algal growth rate. The 72h E_bC_{50} and E_rC_{50} values for cyflumetofen were >0.30 mg/L (initially measured) or >0.0396 mg/L (mean measured). The NOEC for biomass and growth rate were 0.30 mg/L (initially measured) or 0.0396 mg/L (mean measured).

Table 160 Toxicity of cyflumetofen to *Selenastrum capricornutum*

Mean measured concentration [mg a.s./L]	Cell densities after 72 hours [cells x 10 ^{4}/mL]	Mean growth rate (48-72 h)	Reduction (r, growth rates) [%]	Mean area under growth curve (0-72 h)	Inhibition (b, biomass) [%]
Blank-control	92.8	0.05776	-	1728	-
Solvent-control	95.3	0.05825	-0.6	1765	-2.1
0.0396	94.0	0.05786	0.3	1762	0.2

Table 161 The effect of cyflumetofen on mean algal growth rate

Mean measured concentration [mg a.s./L]	Mean Growth rate (0-24 h)	Reduction [%] (0-24 h)	Mean Growth rate (24-48 h)	Reduction [%] (24-48 h)	Mean Growth rate (48-72 h)	Reduction [%] (48-72 h)
Blank-control	0.06610	-	0.06478		0.05776	
Solvent-control	0.06631	-0.3	0.06517	-0.6	0.05825	-0.8
0.0396	0.07076	-6.7	0.06063	7.0	0.05786	0.7

Metabolites

Green algae, *Pseudokirchneriella subcapitata*, (initial cell density 10000 cells/ml of test medium) were exposed to AB-11 (purity 99.6%) at a nominal test concentration of 500 µg/L for 72 hours under static conditions in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of AB-11; acetone was used as solvent carrier. The pH varied between 7.8 and 8.2 and temperature was maintained at 22.7-23.1°C during the test. The measured initial concentration was 493 µg/L; the mean measured concentration was 157 µg/L. AB-11 did not inhibit cell growth or reduce algal growth rate. The 72h E_yC_{50} and E_rC_{50} values for AB-11 were >0.5 mg/L (initially measured) or >157 µg/L (mean measured). The NOEC for yield and growth rate were 0.5 mg/L (initially measured) or 157 µg/L (mean measured).

Table 162 Toxicity of AB-11 to *Pseudokirchneriella subcapitata*

Mean measured concentration [µg/L]	Cell densities after 72 hours [cells x 10 ^{4}/mL]	Yield (y: 0-72 h)	Inhibition yield (0-72 h) [%]	Growth rate (r: 0-72 h)	Reduction growth rates (0-72 h) [%]
Blank-control	94.2	93.15	-	0.06306	-
Solvent-control	104.5	103.49	-	0.06453	-
157	99.7	98.73	4.6	0.06388	1.0

Table 163 The effect of AB-11 on mean algal growth rate

Mean measured concentration [µg/L]	Mean Growth rate (0-24 h)	Reduction [%] (0-24 h)	Mean Growth rate (24-48 h)	Reduction [%] (24-48 h)	Mean Growth rate (48-72 h)	Reduction [%] (48-72 h)
Blank-control	0.05770	-	0.06410	-	0.06740	-
Solvent-control	0.06189	-	0.06198	-	0.06974	-
157	0.06006	3.0	0.05894	4.9	0.07263	-4.1

Green algae, *Pseudokirchenriella subcapitata*, (initial cell density 10000 cells/ml of test medium) were exposed to B-1 (purity 99.99%) at nominal test concentrations of 0.1, 1.0, 10 and 100 mg/L for 96 hours under static conditions (first 24 hours in darkness) in accordance with ISO, EC and OECD guidelines. The test substance was dissolved in test medium. The pH varied between 6.8 and 8.2 and temperature was maintained at 21.8-23.4°C during the test. Measured concentrations were in agreement with nominal concentrations. B-1 did not inhibit cell growth or reduce algal growth rate. The 72h E_yC_{50} and E_rC_{50} values for B-1 were >100 mg/L; the NOEC for yield and growth rate were 100 mg/L.

Table 164 Toxicity of B-1 to *Pseudokirchneriella subcapitata*

Nominal concentration [mg/L]	Cell densities after 96 hours [cells x 10^4/mL]	Yield (y: 0-96 h)	Inhibition yield (0-96 h) [%]	Growth rate (r: 0-96 h)	Reduction growth rate (0-96 h) [%]
Blank-control	253.3	252.31	-	0.06683	-
0.1	209.8	208.80	17.2	0.06832	-2.2
1.0	237.3	236.29	6.3	0.07585	-13.5
10	263.7	262.67	-4.1	0.06520	2.4
100	225.5	224.45	11.0	0.06416	4.0

Table 165 The effect of B-1 on mean algal growth rate

Nominal concentration [mg/L]	Mean Growth rate (24-48 h)	Reduction [%] (24-48 h)	Mean Growth rate (48-72 h)	Reduction [%] (48-72 h)	Mean Growth rate (72-96 h)	Reduction [%] (72-96 h)
Blank-control	0.05912	-	0.07283	-	0.06854	-
0.1	0.07077	-19.7	0.06647	8.7	0.06770	1.2
1.0	0.09092	-53.8	0.06797	6.7	0.06867	-0.2
10	0.06457	-9.2	0.07031	3.5	0.06644	3.1
100	0.05718	3.3	0.06914	5.1	0.06614	3.5

Green algae, *Pseudokirchenriella subcapitata*, (initial cell density 10000 cells/ml of test medium) were exposed to B-2 (purity 99.9%) at a nominal test concentration of 500 µg/L for 72 hours under static conditions in accordance with ISO, EC and OECD guidelines. The tested concentration was limited by the low water solubility of B-2; acetone was used as solvent carrier. The pH varied between 8.1 and 8.2 and temperature was maintained at 21.5-22.9°C during the test. The measured initial concentration in the test was 73 µg/L; the mean measured concentration was 10.1 µg/L. The 72h E_yC_{50} and E_rC_{50} values for B-2 were >73 µg/L (initially measured) or >10.1 µg/L (mean measured). The NOEC for yield and growth rate were 73 µg/L (initially measured) or 10.1 µg/L (mean measured).

Table 166 Toxicity of B-2 to *Pseudokirchneriella subcapitata*

Mean measured concentration [µg/L]	Cell densities after 72 hours [cells x 10^4/mL]	Yield (y: 0-72 h)	Inhibition yield (0-72 h) [%]	Growth rate (r: 0-72 h)	Reduction growth rates (0-72 h) [%]
Blank-control	325.5	324.51	-	0.08034	-
Solvent-control	309.8	308.77	-	0.07965	-
10.1	316.3	315.30	-2.1	0.07991	-0.3

Table 167 The effect of B-2 on mean algal growth rate

Mean measured concentration [µg/L]	Mean Growth rate (0-24 h)	Reduction (%) (0-24 h)	Mean Growth rate (24-48 h)	Reduction (%) (24-48 h)	Mean Growth rate (48-72 h)	Reduction (%) (48-72 h)
Blank-control	0.10001	-	0.07528	-	0.06574	-
Solvent-control	0.09688	-	0.07444	-	0.06762	-
10.1	0.09834	-1.5	0.07507	-0.9	0.06631	1.9

5.4.4   Other aquatic organisms (including sediment)

Toxicological data for cyflumetofen and for one of the metabolites relevant for sediment (AB-1) are available. No further assessment for classification and labelling is done based on this information.

5.5   Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

Summary of the acute toxicity data

No acute L(E)C$_{50}$ values could be derived for cyflumetofen in fish, invertebrates and algae as they are all above the maximum tested concentration and well above its water solubility of 28 µg/L, at 20ºC and pH 7. Maximum tested concentrations were, expressed as nominal/mean measured concentration, 1000/630, 100/63 and 10,000/39.6 µg/L, for fish, invertebrates and algae, respectively.

Summary of the chronic toxicity data

The chronic NOEC for invertebrates (65 µg/L) is < the maximum tested concentration and > water solubility, while the lowest chronic NOECs for fish (54 µg/L; 31d early life stage test) and algae (39.6 µg/L) were the highest tested concentrations, which exceeded the water solubility.

The effect seen in the daphnia reproduction study (mortality at the highest test level; 151 µg/L) was discussed in Section 5.4.2.2 to be difficult to interpret. Especially as reproduction was not affected up to and including the highest test concentration, this effect might not be test material related. In the juvenile growth test with carp increase in body weight, growth rate and body length were significantly affected at the highest test concentration (179 µg/L) being significantly above the water solubility of cyflumetofen. The early life stage toxicity test in fathead minnow showed no effects on body weight, growth rate and body length although this test design is considered to be more sensitive in respect to these endpoints. It can therefore not be confirmed that the effects seen in the juvenile growth test were due to dissolved test material and hence this result will therefore not be taken into account for classification purposes. The NOEC for algae (39.6 µg/L; mean measured concentration) is concluded to be the lowest NOEC for cyflumetofen.

Summary of the relevant metabolites

The metabolites AB-11 and B-2 produce acute EC_{50} values above their maximum solubilities in test medium for the species tested (invertebrates and algae) as well as a chronic NOEC for algae at the maximum solubility in test medium.
Metabolite B-1 produces acute EC_{50} values >100 mg/L for the species tested (invertebrates and algae) as well as a chronic NOEC for algae at 100 mg/L.

Summary of degradation

In order to classify cyflumetofen, its degradation behaviour needs to be considered. As discussed in Section 5.1.3, "rapid degradation" can be demonstrated by ready biodegradability or other evidence of rapid degradation in the environment ($\geq$ 70% abiotic or biotic degradation in the environment in 28 days). Further, it is stated that primary biodegradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

Data on ready biodegradability are not available, but based on the results of the simulation tests (water-sediment studies), cyflumetofen is not expected to be readily biodegradable. Relevant metabolites formed in the water phase are A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8. Three of these metabolites were studied and show no acute aquatic toxicity above their water solubility or 100 mg/L. The other identified metabolites were concluded not to be more toxic than parent cyflumetofen based on QSAR predictions and structural considerations (see Section 5.4). Furthermore, identified cyflumetofen metabolites have BCF values <500 and hence a low potential to bioconcentrate. However, the available QSAR predictions for some endpoints as well as the (lack of) data for unidentified metabolites are concluded not to be sufficiently robust to support a conclusion on "rapid degradation".

CLP- Acute aquatic hazards

According to the criteria of the CLP Regulation, a substance is classified for aquatic acute toxicity if in an aquatic acute toxicity study, an $L(E)C_{50}$ of $\leq$ 1 mg/L is obtained for any of the three trophic levels fish, invertebrates and algae/aquatic plants.

Cyflumetofen is a poorly water-soluble substance, 28 µg/L at 20°C and pH 7. Acute aquatic toxicity is available for all three trophic levels. No effects on aquatic organisms were observed at test concentrations between 39.6 and 630 µg/L, which is above or at the limit of solubility of Cyflumetofen. The available data show that the criteria for classification for aquatic hazard according to Annex I Table 4.1.0 (a) of the CLP regulation are not applicable to cyflumetofen. Therefore, we conclude not to classify the substance for acute aquatic hazard.

CLP – Long-term aquatic hazards

Water solubility of Cyflumetofen is 0.028 mg/L (at 20°C and pH 7) and the lowest NOEC value is 0.0396 mg/L (algae; mean measured concentration). No toxicity is recorded at levels in excess of the water solublilty, therefore the NOEC for classification purposes may be considered to be greater that the measured water solubility. According to the guidance in such cases, consideration should be given to whether the category Chronic 4 should apply. Cyflumetofen is considered as not rapidly

degradable however the experimental BCF value does not exceed the criteria threshold of 500 L/kg. Therefore, it is concluded not to classify cyflumetofen for long-term aquatic hazards.

CLP – Hazardous to the ozone layer

Any substances having an Ozone Depleting Potential (ODP) greater or equal to 0.005 should be classified as hazardous to the ozone layer.
Although no ODP is available for cyflumetofen, its short half-life in air and low potential for volatilization does not support classification as hazardous to the ozone layer.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Cyflumetofen needs not to be classified for environmental hazards.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Cyflumetofen is a new active substance for plant production products (specifically an acaricide used in both indoor and outdoor spray applications on ornamental crops, nursery trees, perennial ornamentals and public greens for the control of *Tetranychyus urticae* (red spider mite)). It was approved under Regulation (EC) No 1107/2009 via Commission Implementing Regulation (EU) No 22/2013 and subsequently amending the Annex to Commission Implementing Regulation (EU) No 540/2011. The substance currently has no harmonised classification. The Dossier Submitter proposed not to classify the substance as hazardous to the aquatic environment.

Degradation

A hydrolysis study according to JMAFF, EPA N:161-1, EU 95/36/EC, FAO (1993) and in compliance with GLP was run at pH 4, 5, 7 and 9 at 25 °C and at a concentration of 0.1 mg/L. Cyflumetofen was susceptible to hydrolysis in acidic, neutral and basic buffer solutions, the hydrolysis rate increasing with increasing pH. Hydrolytic half-lives of cyflumetofen at 25 °C are 7.7 d (pH 4), 6.0 d (pH 5), 9.8 hrs (pH 7) and 10.3 min (pH 9). At environmentally relevant pH values (pH 5-7), five hydrolysis products exceeding 10% of applied radioactivity were identified (A-1, A-2, A-18, AB-1 and B-1).

The photodegradation of radio-labelled cyflumetofen in water was studied according to JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993). The study, in compliance with GLP, was carried out at 25 °C with continuous artificial light. The half-life was 1.28h at pH 5 (aqueous buffer solution) and 1.07 h in natural water, indicating that cyflumetofen is susceptible to aquatic photolysis. Three degradation products which account for > 10% were identified in aqueous buffer solution (B-1, AB-7 and AB-15), in natural water AB-1 was also observed in significant amounts.

There is no screening test for biodegradability available.

Two water/sediment studies were performed with a ^{14}C label in either of the two aromatic

rings of cyflumetofen (A-ring and B-ring). These studies, carried out according to OECD TG 308 and in compliance with GLP, used the same two water/sediment systems; the first one was run for 57 and 98 days, the second one for 103 days. Both studies showed that cyflumetofen partitions from the water phase to the sediment and exhibited very low to moderate retention in the whole system (DT_{50} values range from 0.08 to 14 days). Mineralisation to CO_2 was a minor process (2-3%) except in the A-label study where 20% mineralisation was observed in one of the two test systems. Bound residues accounted for between 13 to 33% AR. Several relevant degradation products (>10% of applied radioactivity) were formed in both compartments (AB-11, B-1) or in the water compartment only (A-2, Met-1, Met-8) and in the sediment compartment only (AB-1, Met-4). Metabolite B-2 was formed in the water/sediment study with B-radiolabelled cyflumetofen only. Two metabolites were not identified (Met-4 and Met-8). Where possible, DT_{50} system values were calculated for metabolites (range from 0.77 to 320 days).

Based on the results of the simulation tests, the dossier submitter considered cyflumetofen as not rapidly degradable since it appeared susceptible for primary degradation but not ultimate mineralisation. Due to missing information for all degradation products, it could not be demonstrated that they do not fulfil the criteria for classification as hazardous to the aquatic environment.

Bioaccumulation

A fish bioconcentration study was available for cyflumetofen, carried out according to OECD TG 305 and in compliance with GLP. Carp (*Cyprinus carpio*) were exposed to B-[ring-U-^{14}C] cyflumetofen for 21 days in a flow-through system, followed by 32 days of depuration in clean water. Nominal concentrations of 1.0 and 10 µg/L, plus solvent control were tested in one replicate aquarium containing 48 fish at test initiation (22 fish were used for the solvent-control).

BCF values for total radioactivity in whole fish were 170 and 146 L/kg wwt at 1.0 and 10 µg a.s./L respectively (lipid BCF normalised to 1% fat 34 and 29 L/kg wwt). Since cyflumetofen was not detected in any fish sample, the BCF for cyflumetofen was <100 L/kgat both exposure levels.

No measured bioaccumulation data are available for the seven major metabolites identified to be relevant for surface water (A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8). For some of them (B-2, AB-7, AB-11), despite the estimated Log K_{OW} values indicate a potential for bioaccumulation, the bioconcentration was not considered to be of concern due to their short half-lives in water. No assessment was made for unidentified metabolites Met-1 and Met-8.

Aquatic toxicity

Summary of relevant information on aquatic toxicity performed with cyflumetofen and for three of the relevant aquatic degradation products AB-11, B-1 and B-2:

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [a] [mg/L]	Remarks
Acute toxicity to fish							
ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Oncorhynchys mykiss* (rainbow trout)	96h (flow-through)	Survival, LC_{50}	>0.63 (mm)	Migchielsen, 2003a, (IIA 8.2.1.1/01) [d]

ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Cyprinus carpio* (carp)	96h (flow-through)	Survival, LC50	>0.54 (mm)	Migchielsen, 2003b (IIA 8.2.1.1/02) [d]
Acute toxicity to invertebrates							
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.0%	*Daphnia magna*	48h (flow-through)	Immobility, EC$_{50}$	>0.063 (mm)	Bouwman, 2003a (IIA 8.3.1.1/01) [d]
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.4%	*Daphnia magna*	48 h (flow-through)	Immobility, EC$_{50}$	0.70 (im) [f]	Migchielsen 2009b (IIA 8.3.1.1/02) [d]
ISO 6341; EEC C.2; OECD 202	AB-11	99.6%	*Daphnia magna*	48h (static)	Immobility, EC$_{50}$	>0.5 (nom) or >0.476 (mm)	Migchielsen, 2009a (IIA 8.3.1.1/03) [d]
ISO 6341; EEC C.2; OECD 202	B-1	99.99%	*Daphnia magna*	48h (static)	Immobility, EC$_{50}$	>180 (nom)	Migchielsen, 2008b (IIA 8.3.1.1/04) [d]
ISO 6341; EEC C.2; OECD 202	B-2	99.9%	*Daphnia magna*	48h (static)	Immobility, EC$_{50}$	>0.039 (im) or >0.0062 (mm)	Bouwman, (2009a) IIA 8.3.1.1/05)
Acute toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate, EC50	>0.30 (im) or >0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, EC50	>0.5 (nom) or >0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneriella subcapitata*	96h (static)	Yield/growth rate, EC50	>100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/growth rate, EC50	>0.073 (im) or >0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]
Chronic toxicity to fish							
OECD 215	cyflumetofen	98.4%	*Cyprinus carpio* (carp)	28d (flow-through)	Survival/growth, NOEC	0.072 (mm)	Migchielsen, 2007a (IIA 8.2.3/01) [d]
OECD 212	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	8d (flow-through)	Survival/hatching, NOEC	≥ 0.145 (mm)	Migchielsen, 2008a (IIA 8.2.4/01) [d]
OECD 210	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	31d (flow-through)	Hatching/larval survival/growth, NOEC	0.054 (mm)	Migchielsen, 2010 [e]
Chronic toxicity to invertebrates							
OECD 211; ISO International	cyflumetofen	98.4%	*Daphnia magna*	21d (flow-through	Mortality, NOEC	0.065 (mm) [b]	Migchielsen, 2007b (IIA 8.3.2.1/01) [d]

Standard 10706:2000 (2000-03-30)							
Chronic toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate, _	>0.30 (im) or >0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneri ella subcapitata*	72h (static)	Yield/growth rate, NOEC [c]	>0.5 (nom) or >0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneri ella subcapitata*	96h (static)	Yield/growth rate, NOEC [c]	>100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneri ella subcapitata*	72h (static)	Yield/growth rate, NOEC [c]	>0.073 (im) or >0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]
Sediment dwelling organisms							
OECD 219	cyflumetofen	98.4%	*Chironomus riparius*	28d (static)	Emergence/development (water spiked), NOEC	≥ 0.064 (im)	Desmares-Koopmans, 2009a (IIA 8.5.2/01) [d]
OECD 218	AB-1	99.8%	*Chironomus riparius*	28d (static)	Emergence/development (sediment spiked), NOEC	59.6 mg/kg (im)	Desmares-Koopmans, 2009b (IIA 8.5.2/02) [d]

[a] (mm): mean measured concentrations; (nom): nominal; (im): initially measured

[b] This study is less reliable due to high mortality in the control. However, the experts in the Pesticides Peer Review Expert Meeting considered that a new chronic study with daphnids for cyflometofen is not required based on the following arguments: in the study, no effects were seen on reproduction (thus: NOECreproduction ≥151 µg a.s./L); the chronic NOEC for daphnids of 65 µg a.s./L is based on mortality which is a worst case approach; the NOEC of 65 µg a.s./L is comparable to the acute NOEC for daphnids.

[c] The NOEC was taken from the original study report as this endpoint was not reported in the EFSA evaluation and the DAR.

[d] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B.9; September 2011.

[e] This study (Migchielsen, 2010: Fish early-life stage toxicity test with OK-5101 (flow-through), NOTOX B.V., 's-Hertogenbosch, The Netherlands. Unpublished report No. 487052) was submitted as supplement to the DAR. Note:"OK-5101" is used as code for "cyflumetofen".

Toxicity to Fish

The acute toxicity of cyflumetofen (purity 98.0%) for *Oncorhynchys mykiss* (rainbow trout) and *Cyprinus carpio* (Common carp) was tested in a 96h flow-through study in accordance with ISO 7346-3;EEC C.1;OECD TG Guideline 203, (Migchielsen, 2003a and b). Juvenile organisms were exposed to a nominal concentration of 1.0 mg of the test

substance/L, using acetone as solvent carrier. The mean measured concentrations was 0.63 mg cyflumetofen/L for the rainbow trout test, and 0.54 mg cyflumetofen/L for the carp test, in both cases exceeding the water solubility of cyflumetofen (reported to be 0.028 mg/L). During the tests, small amounts of precipitate and a thin floating layer were observed in the solution with cyflumetofen but this was reported not to have affected the fish. Symptoms of toxicity were noted in rainbow trout exposed to cyflumetofen within 24 hours of exposure and included slower swimming and discolouration. The LC_{50} was > 0.63 mg/L. No mortality or symptoms of toxicity were noted in the carp exposed to cyflumetofen. The LC_{50} was > 0.54 mg/L.

For the chronic toxicity of cyflumetofen, three studies of Migchielsen, 2007a 2008a and 2010 (see table above) were reported. RAC notes that the Migchielsen, 2007a and 2008a studies are extracted from the revised DAR report (Sep. 2011), whilst the most recent data provided (Migchielsen, 2010) refers to a supplement document to the DAR, not currently available to RAC.

In the juvenile growth test with carp (Migchielsen, 2007a, OECD TG 215) the nominal concentrations were 10, 22, 46, 100 and 220 µg/L in a flow-through test design for 28 days. Mean measured concentrations in the test were 7.2, 16, 34, 72 and 179 µg/L. Increases in body weight, growth rate and body length were significantly different from control (at 5% level, according to DAR, 2011) at the highest mean measured concentration of 179 µg/L, above the water solubility of cyflumetofen. Therefore, the related NOEC was 0.072 mg/L (mean measured concentration).

In the Migchielsen (2008a) test (OECD TG 212), fathead minnow was exposed at nominal concentrations of 10, 46 and 220 µg/L in a flow-through test design for 8 days. Mean measured concentrations in the test were 5.9, 27 and 145 µg/L. The only observed effect was for the larval survival at the highest mean measured concentration 145 µg/L, but the slight reduction was not statistically significant (see section "additional information"). Therefore, the NOEC was ≥ 145 µg/L.

The early life stage toxicity test in fathead minnow (Migchielsen, 2010, OECD TG 210) showed no effects on hatching success, larval survival, body length and body weight at any tested concentration (nominal) of 15, 30 and 60 µg/L in a flow-through test design for 31 days. Mean measured concentrations were 11, 34 and 54 µg/L. Therefore, the NOEC was the highest tested concentration of 54 µg/L (mean measured), exceeding the water solubility.

No information is available for the metabolites AB-11 and B-2 and Metabolite B-1.

Toxicity to Aquatic invertebrates

Two acute toxicity tests of cyflumetofen for *Daphnia magna* and three for the main metabolites were reported (see table above). All tests were according to OECD TG 202 without any toxic effects up to the water solubility levels.

For the chronic toxicity of cyflumetofen, juvenile *Daphnia magna* (Migchielsen, 2007b) were exposed to cyflumetofen at nominal concentrations of 10, 22, 46, 100 and 220 µg/L in a flow-through test for 21 days, in accordance with ISO and OECD TG 211 guidelines. Mean measured concentrations in the test were 7.5, 12, 32, 65 and 151 µg/L, respectively. Mortality at 151 µg/L was significantly different from that in the solvent-

control after 20 days. Parental body length and reproduction was not affected at any concentration. Under flow-through conditions, the 21-day NOEC of cyflumetofen to *Daphnia magna* was 65 µg/L, based on a significant effect on parental survival after 20 days.

The mortality in the blank control (58%) and in the solvent control (28%), of the study was above the validity criterion of the guideline of ≤ 20%. Since the study does not fulfil the validity criterion of the relevant guidelines, the reliability of the study was discussed by the experts in the Pesticides Peer Review Expert Meeting. It was concluded that: […] *in the study, no effects were seen on reproduction (thus: NOEC$_{reproduction}$ ≥ 151 µg/L); the chronic NOEC for daphnids of 65 µg/L is based on mortality which is a worst case approach; the NOEC of 65 µg/L is comparable to the acute NOEC for daphnids*.

<u>Toxicity to algae</u>

Cyflumetofen (purity 98.0%) was tested for 72 hours under static conditions on the green algae *Selenastrum capricornutum*, in accordance with ISO 8692;EEC C.3; OECD TG 201 (Bouwman, 2003b). The substance was dissolved in acetone because of the low water solubility (i.e. 0.028 mg/L). The initial measured concentration of 0.30 mg/L exceeded the water solubility of cyflumetofen. The mean measured concentration in the test, calculated with geometric means, was 0.0396 mg/L. Cyflumetofen did not reduce algal growth rate so the 72h ErC$_{50}$ values were > 0.30 mg/L (initially measured) or > 0.0396 mg/L (mean measured). The NOEC for growth rate was > 0.30 mg/L (initially measured) or > 0.0396 mg/L (mean measured).

The three metabolites AB-11, B-1 and B-2 were tested on *Pseudokirchneriella subcapitata* and the studies were performed in accordance with ISO 8692; EEC C.3; OECD TG 201.

The green algae was exposed for 72 hours to the metabolite AB-11 (purity 99.6%) dissolved in acetone (Migchielsen, 2009b). No reduction in algal growth rate was observed, and the following values were established: ErC$_{50}$ > 0.493 mg/L (initially measured) or > 0.157 mg/L (mean measured); NOEC for growth rate were > 0.493 mg/L (initially measured) or > 0.157 mg/L (mean measured).

For the metabolite B-1, the nominal test concentrations of 0.1, 1.0, 10 and 100 mg/L were used, the measured concentrations were in agreement with them (Migchielsen, 2008c). The test was performed for 96 hours under static condition and no reduction in algal growth rate was observed at the higher concentration. The 72h ErC$_{50}$ value for B-1 was > 100 mg/L; the NOEC for yield and growth rate was > 100 mg/L.

Pseudokirchenriella subcapitata, (Bouwman, 2009b) was exposed to B-2 (purity 99.9%) for 72 hours under static conditions; acetone was used as a solvent carrier. B-2 did not reduce algal growth rate, the 72h ErC$_{50}$ values were > 0.073 mg/L (initially measured) or > 0.0101 mg/L (mean measured). The NOEC for growth rate were > 0.073 mg/L (initially measured) or > 0.0101 µg/L (mean measured).

Comments received during public consultation

Three MSCAs and two stakeholders commented on ENV classification. Only one MS did not agree with the proposal of no classification for environmental hazards and proposed classification and labelling for long-term aquatic hazard with category Chronic 1; H410.

The reasoning for the disagreement was the presence of a chronic toxicity study on fish

(Salinas, 2001, as reported in EFSA Journal 2016; 14(11):4635) which reported a NOEC value of 0.0292 mg a.s./L (mean measured). This value was considered by the MSCA as equal to the water solubility of 0.028 mg/L (at 20°C and pH 7), proposing a classification for long-term aquatic hazard as Aquatic Chronic 1; H410.

According to the Dossier Submitter's (DS) response, the Salinas (2011) study is relevant and valid, but it does not change the proposed no classification for chronic toxicity. Indeed, the DS pointed out that the toxicity value reported in the EFSA Journal was ≥ 0.0292 mg/L because no effects (to hatching success, survival or growth of the fathead minnow) were observed at the tested concentration which should be regarded as close to the water solubility limit (EFSA Journal, 2016). Water solubility is reported as 28 µg/L at 20 °C and pH 7.

Moreover, the DS reported that "EFSA *considers it more appropriate to remove the measured concentrations which exceeded the water solubility in the calculation of the exposure value. However, this does not affect the overall conclusion of the study i.e. that there were no effects at the tested concentration which should be regarded as close to the water saturation level.*"

One MS commented on the acute toxicity test of metabolite B2 to *Daphnia magna* (Bouwman, 2009a) saying that for rapidly hydrolysing substances it is considered justified by the DS to base the toxicity endpoint on the measured initial concentration. Referring to the CLP guideline (I.4.1) recommendation, the commenting MSCA reminded that for unstable substances, the geometric mean concentration at the start and the end of test for classification purposes should be considered. RAC noted that for the metabolite B-2, the concentration of the start was 0.039 mg/L and at the end of test the concentration resulted below the analytical detection limit (0.002 mg/L). Therefore, according to CLP guideline Version 5.0 – July 2017 (I.4.1) the $L(E)C_{50}$, should be calculated based on the geometric mean concentration and half of the detection limit. The result is = 0.0062 mg/L, as reported in the DAR (Sept. 2011) and in the CLH report (Table 147).

Additional key elements

Key elements

Regarding chronic toxicity to fish, RAC highlighted that the relevance of additional information cited by the commenting MSCAs and the DS in the RCOM should be evaluated for environmental classification. In particular, there was a mention of an additional study by Salinas (2011), an ELS test over 34 days under flow-through conditions with *Pimephales promelas* performed according to OECD TG 210, which gave a NOEC value (survival, hatching, growth) of 0.0292 mg a.s./L (mean measured).

RAC notes that on the public EFSA website, the documents on Peer review of the pesticide risk assessment (EFSA Journal, 2016), and on the outcome of the consultation on confirmatory data (EFSA supporting publication 2016/EN/997) are available. Based on the available information, RAC agrees with the conclusion of the DS that there were no effects at the tested concentration, and therefore no effects close to the water solubility limit.

A further issue concerns the statistical significance of the observed effect for the larval survival at the highest mean measured concentration 145 µg/L, in the test (OECD TG

212) on fathead minnows (Migchielsen, 2008a). Indeed, in the DAR document (EFSA Sept. 2011) provided by the DS, further information on the statistics was reported. As requested by EFSA during peer review: *Data were confirmed to be normally distributed by the Chi-square test for normality. Data for the two controls were tested with the F-test for equality of variances followed by ANOVA and a two-sample t-test. As the controls were found not to differ significantly, they were pooled for the subsequent analysis. Data were analysed by the Chi-square test for normality and the Levene's test for homogeneity of variance, followed by ANOVA and Dunnett's t-test, Bonferroni t-test and Tukey test (a=0.05, two-sided). There were no statistically significant effects at any test concentration. The analysis was also performed without pooling of the control data, using the same tests and comparing the test groups with the solvent control. No statistically significant differences were found. The NOEC was ≥ 145 µg a.s./L.*

B.9.2.1.2-2   Toxicity of OK-5101 to egg and sac-fry stages of fathead minnow under flow-through conditions

Mean measured concentration [µg a.s./L]	Egg survival after 24 hours [%]	Hatching rate [%]	Survival of eggs and larvae after 8 days[%]
Blank-control	93	61	93
Solvent-control	93	100	80
5.9	97	100	90
27	100	100	97
145	87	100	73

Assessment and comparison with the classification criteria

Degradation

RAC agreed with the DS proposal to consider cyflumetofen as not rapidly degradable. The substance is not hydrolytically and photochemically stable, data on ready biodegradability are not available and the results of water/sediment simulation studies showed that cyflumetofen is susceptible for primary degradation ($DT_{50} < 16$ days), but it did not ultimately degrade to a level greater than 70% within 28 days. Annex I, section 4 of the CLP regulation states that rapid degradation can be demonstrated by ready biodegradability or ether evidence of rapid degradation in the environment (≥ 70% abiotic or biotic degradation within 28 days). Furthermore, it states that primary degradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

For cyflumetofen, even if some of the relevant metabolites formed in the water-phase can be considered not hazardous to the aquatic environment, the lack of data regarding the unidentified metabolites are sufficient to conclude that cyflumetofen is not rapidly degradable.

Bioaccumulation

The measured BCF (for total radioactivity) in whole fish of 170 - 146 L/kg wwt is below the CLP criterion (BCF ≥ 500). Therefore, RAC agrees with the DS that the bioaccumulation potential of cyflumetofen is low.

Acute aquatic hazards

Acute aquatic toxicity data is available for all three trophic levels. The water solubility of cyflumetofen is 0.028 mg/L (at 20°C and pH 7). No effects on aquatic organisms were observed at test concentrations below or equal to the water solubility limit of cyflumetofen.

Therefore, RAC agrees with the DS's proposal **not to classify cyflumetofen for acute aquatic hazards**.

Chronic aquatic hazards

Chronic aquatic toxicity data is available for all trophic levels and the lowest NOEC value provided by the DS is 0.0396 mg/L (algae; mean measured concentration).

The available information shows no toxicity below or equal to the water solubility limit, therefore the NOEC for classification purposes was considered greater than the measured water solubility (0.028 mg/L at 20°C and pH 7). Although cyflumetofen is considered not rapidly degradable, the experimental BCF value does not exceed the CLP criterion threshold of 500 L/kg, so classification as Aquatic Chronic 4 is not warranted.

Therefore, RAC agrees with the DS's proposal **not to classify cyflumetofen for chronic aquatic hazards**.

6 OTHER INFORMATION

None

7 REFERENCES

Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Volume 3, Annex B; October 2010

Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, addendum September 2011

Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, addendum October 2011

ECBI/08/04 Rev.2. EU Commission Group of Specialised Experts in the fields of carcinogenicity, mutagenicity and reprotoxicity: Leydig tumours 2004, ECBI/08/04 Rev. 2, 2004. http://ecb.jrc.ec.europa.eu/documents/Classification-Labelling/GUIDANCE_DOCUMENTS/0804r2_sr_SE_01_2004_LCT_excerpt.pdf

EFSA (2011). Peer Review Report on Cyflumetofen, 12 December 2011

EFSA (2012). Conclusion on the peer review of the pesticide risk assessment of the active substance cyflumetofen. EFSA Journal 2012;10(1):2504

European Commission (2012). Review Report for the active substance cyflumetofen. SANCO/12618/2012, 25 October 2012.

Hayashi N., Sasama Y., Takahashi N., Ikemi N. (2013). Cyflometofen, a novel acaricide – its mode of action and selectivity. Pest Manag Sci 2013; 69: 1080–1084

A reference list for all individual studies and references is added separately in a confidential document and will be included in the IUCLID file.

Additional references

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment for the active substance cyflumetofen in light of confirmatory data. EFSA Journal 2016;14(12):4635, 20 pp. doi:10.2903/j.efsa.2016.4635
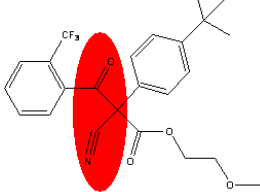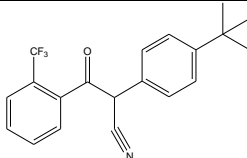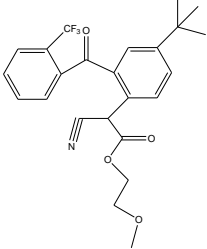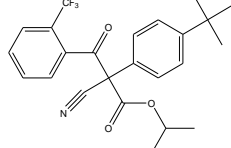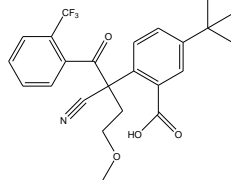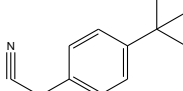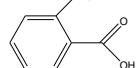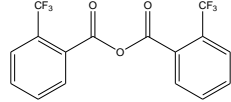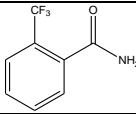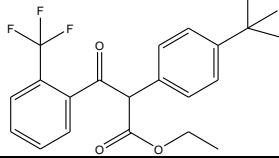
EFSA (European Food Safety Authority), 2016. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for cyflumetofen in light of confirmatory data. EFSA supporting publication 2016:EN-997. 25 pp.

8 ANNEXES

8.1 Relevance of the tested batches

Documentation on the relevance of the tested batched is added separately in a confidential document (volume 4 of the DAR and Pluijmen, 2014).

8.2   Structural formulars

Structure	Name	Matrix and max. level of formation (%)	Mw	logPow
	cyflumetofen (OK-5101) presumed active site is indicated by shaded area		447.5	4.3[(A)] 4.69[(B)] 6.18[(C)]
	AB-1	Sediment: 15 Soil: 22	345.4	5.33[(B)] 5.46[(C)]
	AB-7	Water: 11 (photolysis)	447.5	4.91[(B)] 5.00[(C)]
	AB-11	Water: 10 Sediment: 10	431.5	5.87[(B)] 7.32[(C)]
	AB-15	Water: 55 (photolysis)	447.5	5.05[(B)] 5.87[(C)]
	A-2	Water: 18	173.3	3.47[(B)] 3.14[(C)]
	B-1	Water: 65 Sediment: 22 Soil: 63 Plant leaves: 9.1	190.1	2.49[(B)] 2.87[(C)]
	B-2	Water: 15 Sediment: 28	362.2	4.26[(B)] 4.96[(C)]
	B-3	Soil: 23	189.1	0.97[(B)] 0.68[(C)]
	Metabolite 1 (tentative structure)	Water: 10.7	392.4	6.21[(B)]
Unidentified	Metabolite 4	Sediment: 10.7	-	-
Unidentified	Metabolite 8	Water: 19.5	-	-

(A) Experimental value
(B) Estimated with EPA Epi Suite software
(C) Estimated with ACD-Labs-LogP