# 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**

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

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# 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<br>Carc. 2; H351 |
| **Resulting harmonised classification (future entry in Annex VI, CLP Regulation)** | Skin Sens. 1A; H317<br><br>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 Skin Sens. 1A (H317: May cause an allergic skin reaction).
- 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 Carc 2 (H351: Suspected of causing cancer) 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<br>GHS09<br>Wng | | | 23 |
| Acute Tox. 4 | H332 | H332 | | | | | |
| Aquatic Acute 1 | H400 | H400 | | | | | |
| Skin Sens. 1 | H317 | H317 | | GHS07<br>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).

# 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

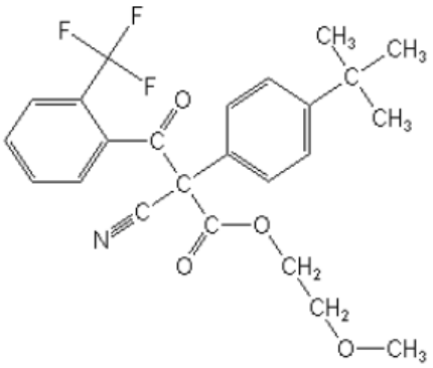
### 1 IDENTITY OF THE SUBSTANCE

### 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])<br><br>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%):<br>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%).<br>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%)<br>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%):<br>acetone: >500 g/L solvent<br>dichloromethane:<br>>500 g/L solvent<br>ethyl acetate: >500 g/L solvent<br>n-hexane: 5.16 g/L solution<br>methanol: 98.7 g/L solution<br>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;<br>DTA/TGA (open vessel)<br>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.

# 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 Cmax of 0.95-1.4 mg eq/L was reached after 1 hour (low dose) and the Cmax 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 $_{0\rightarrow\infty}$ [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 ($AUC_{0\rightarrow\infty}$) 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).

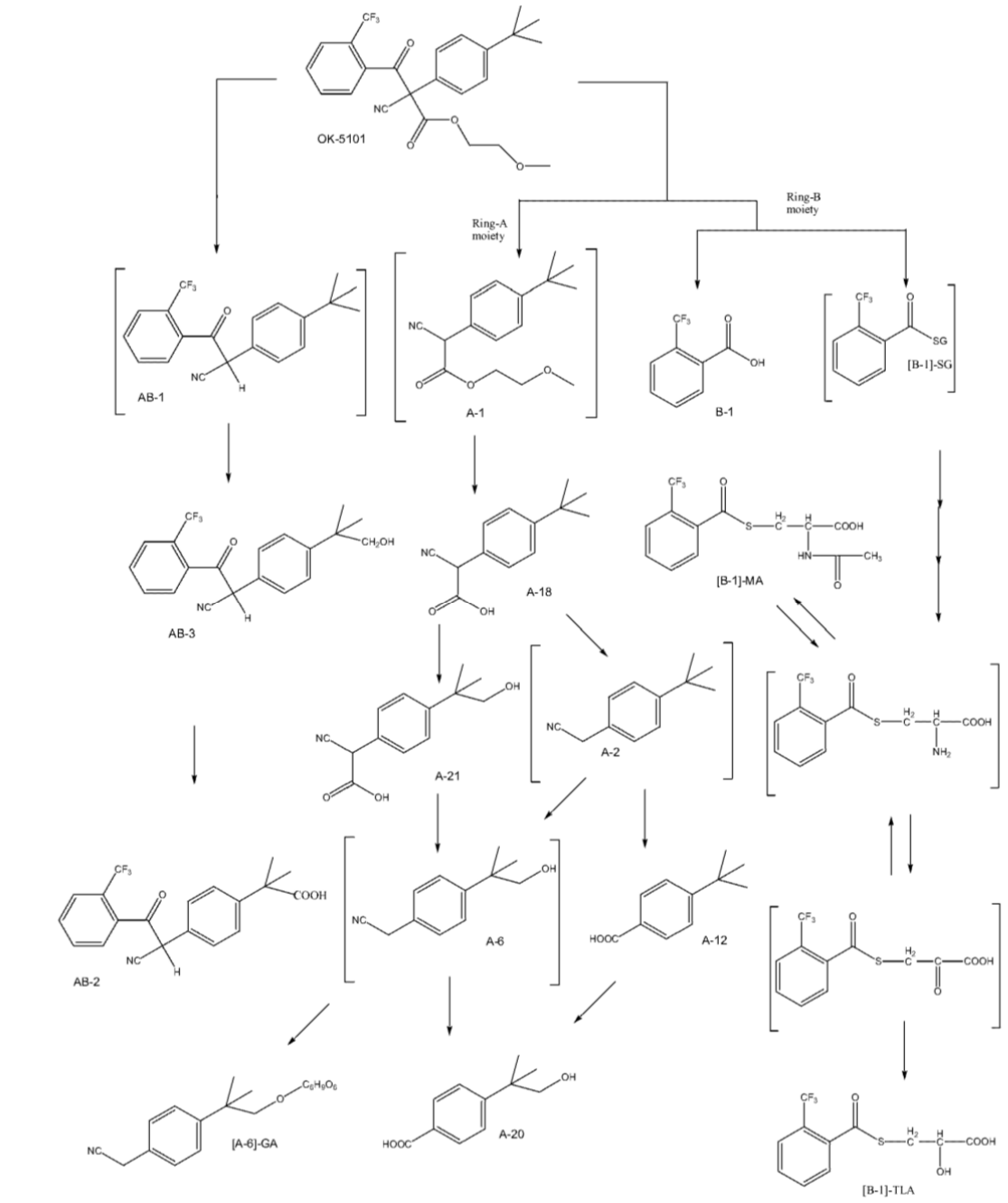
Also identified at levels >1% AR were:

- 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.




GA: glucuronic acid conjugate; SG: glutathione conjugate
MA: mercapturic acid conjugate; TLA: thiolactic acid conjugate
[ ] = possible intermediate

**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 $AUC_{0\rightarrow\infty}$ 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.

## 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.

## 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 $cm^2$ |
| 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.

### 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.

### 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<br>50% topical induction<br>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<br>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.

### 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.

### 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<br><br>OECD 407, rat, Fischer (F344/DuCrj)<br><br>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<br><br>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)<br><br>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<br><br>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<br><br>OECD 407, rat, Fischer (F344/DuCrj)<br><br>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<br><br>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<br><br>OECD 407, mouse, ICR (Crj :CD-1)<br><br>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<br><br>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 |

| Method | Results | Remarks | Reference |
|---|---|---|---|
| 28 day oral (gelatin capsule) toxicity study, dog, beagle (no specific OECD guideline)<br><br>0, 100, 300 or 1000 mg/kg bw/d | NOAEL: 100 mg/kg bw/d<br><br>LOAEL; 300 mg/kg bw/day | Vacuolation of adrenal cortical cells, increased adrenal weight | [a]Nagashima, 2003a |
| 13 week oral (dietary) toxicity study OECD 408, rat, Fischer (F344/DuCrj)<br><br>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<br><br>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)<br><br>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<br><br>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<br><br>0, 30, 300 or 1000 mg/kg bw/d | NOAEL: 300 mg/kg bw/d<br><br>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<br><br>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<br><br>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<br>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<br>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);<br>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<br><br>0, 100, 300 or 1000 mg/kg bw/d | NOAEL >1000 mg/kg bw/day<br><br>LOAEL: - | No adverse effects | [c]Buesen R. et al. 2010a |

| Method | Results | Remarks | Reference |
|---|---|---|---|

[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<br>< 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**<br>-solid perineal region | 0/6 | 0/6 | 0/6 | 0/6 | 0/6 | 6/6 | |
| **Body weight** | | | | | | dc | |
| **Food consumption** | | | | | | dc | |

| **Dose (mg/kg food)** | **0** | | **1000** | | **10000** | | **dr** |
|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | |
| **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 | |
| **Urinalysis** | No treatment-related changes | | | | | | |
| **Organ weights** | | | | | | | |
| - thymus | | | | | $dc^{a,r}$ | | |
| - liver | | | $ic^{r}$ | | $ic^{a,r}$ | $ic^{a,r}$ | m |
| - spleen | | | | | | $dc^{a}$ | |
| - kidneys | | | | | $ic^{r}$ | $ic^{r}$ | |
| - adrenals | | | $ic^{r}$ | $ic^{a,r}$ | $ic^{a,r}$ | $ic^{a,r}$ | m,f |
| - testis | | | | | $ic^{r}$ | | |
| **Pathology** | | | | | | | |
| macroscopy | | | | | | | |
| -adrenal, hypertrophy | 0/6 | 0/6 | 0/6 | 5/6 | 6/6 | 6/6 | m,f |
| microscopy | | | | | | | |
| **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 |

| | Males | | | Females | | |
|---|---|---|---|---|---|---|
| Dose level [ppm ] | 0 | 1000 | 1000 | 0 | 1000 | 1000 |
| * 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<br>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 |

| **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** |
| | 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 | |
| **Clin. Chemistry** | | | | | | | | | | | |
| - ALP | | | | | | | | dc | dc | | |
| - BUN | | | | | | | | | ic | | |
| - total cholesterol | | | | | | | dc | | dc | dc | |
| - triglycerides | | | | | | | dc | | dc | d | m |
| - potassium | | | | | | | | | 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 |
| 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 |

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

**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 |

| 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 | 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 |
| | 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**** |

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

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)<br>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**<br>- platelet count (%) | | | 100 | 87 | 94 | 99 | 97 | 93 | 94 | 86* | |
| **Clin. Chemistry**<br>- BUN | | | | | | | | | | i | |
| **Organ weights**<br>- thyroids | | | $d^{a,r}$ | | $d^{a,r}$ | | $d^{a,r}$ | | $d^{a,r}$ | | |

| **Dose (mg/kg food)** | **0** | | **100** | | **500** | | **1000** | | **5000** | | **dr** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | |
| - lungs | | | | dc[a,r] | | | | | | dc[a,r] | |
| - spleen | | | | | | | dc[a], | | | | |
| - adrenals | | | | | | | d[r] | | i[a,r] | i[a,r] | |
| **Pathology** | | | | | | | | | | | |
| macroscopy | no treatment-related findings | | | | | | | | | | |
| 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 |

| 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 | 4.6 | 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 |
| | 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 |

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

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) | 4.0 | 4.3 | 4.4 | 4.3 | 4.7* | 4.3 | 4.6* | 4.6 | |
| - calcium (mg/dL) | 10.3 | 10.8 | 10.9 | 10.8 | 11.0* | 10.8 | 11.0* | 10.7 | |
| - total bilirubin (mg/dL) | 0.10 | 0.09 | 0.06 | 0.09 | 0.05* | 0.09 | 0.07 | 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 atrioventricular valve, discoloured red foci | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 0/3 | 1/3 | |
| microscopy | | | | | | | | | |

| Dose (mg/kg bw) | 0 | | 100 | | 300 | | 1000 | | dr |
|---|---|---|---|---|---|---|---|---|---|
| | m | f | m | f | m | f | m | f | |
| 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)<br>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 | | | | | | | ic | ic | | | |
| - grip strength forelimb | | | | | dc | | | | | | |
| **Body weight** | no treatment-related findings | | | | | | | | | | |
| **Food consumption** | no treatment-related findings | | | | | | | | | | |
| **Ophthalmoscopy** | no treatment-related findings | | | | | | | | | | |

| Dose (mg/kg food) | 0 | | 100 | | 300 | | 1000 | | 3000 | | dr |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | m | f | m | f | m | f | m | f | m | f | |
| **Haematology** | | | | | | | | | | | |
| - prothrombin time | | | | | | | | | ic | | |
| - total leukocyte count | | | | | | | | dc | | dc | |
| - myeloid/erythroid | | | | d | | d | | dc | | d | |
| **Clin. Chemistry** | | | | | | | | | | | |
| - globulin | | | | | | | | dc | | dc | |
| - A/G ratio | | | | | | | | ic | | ic | |
| **Urinalysis** | | | | | | | | | | | |
| - ketones | | | | | dc | | | | | | |
| **Organ weights** | | | | | | | | | | | |
| - liver | | | | | | | ic[r] | | ic[a,r] | ic[r] | |
| - kidneys | | | | | | | | | ic[r] | ic[r] | |
| - adrenals | | | | | | | | ic[r] | | ic[a,r] | |
| - heart | | | | | dc[a] | | | | dc[a] | | |
| **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 ≤ 0.05; ** p ≤ 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 ≤ 0.05; ** p ≤ 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)<br>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** | | | | | | | | | | | |
| - 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 |
| | 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 |

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

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] | |

| **Dose (mg/kg bw)** | **0** | | **30** | | **300** | | **1000** | | **dr** |
|---|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | |
| **Urinalysis** | | | | | | | | | |
| - occult blood | | | | | | 1/4[1] | | 1/4[1]<br>1/4[2] | |
| - erythrocytes | | | | | | 1/4[1] | | 1/4[1]<br>1/4[2] | |
| **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 |
| | 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 |

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

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) ($x10^2/\mu L$) | 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
$p \leq 0.05$; ** $p \leq 0.01$

**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<br>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)<br>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** | | | | | | | | | | | |
| <u>macroscopy</u> | no treatment-related findings | | | | | | | | | | |
| <u>microscopy</u><br>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 |

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

**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 |

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

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

| | | | | | |
|---|---|---|---|---|---|
| reference | : | Yoshida, T., 2012 | exposure | : | Main groups: 12 months, diet<br>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 groups: 30/sex/dose | **NOAEL** | : | <250 mg/kg bw/d (M)<br><319 mg/kg bw/d (F) |

[1] corresponding to250 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** | **MCV** | **MCH** | **PLT** | **Fib** | **Retics** | **PT** |
|---|---|---|---|---|---|---|---|---|
| | | **[$10^{12}$/L]** | **[fl]** | **[fmol]** | **$10^3$/µL** | **mg/dl** | **%** | **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** | **T.Chol** | **TG** | **T.Bil** | **K** |
|---|---|---|---|---|---|---|
| | | **mg/dl** | **mg/dl** | **mg/dl** | **mg/dl** | **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 |

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

**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.

## 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)<br>21-5000 µg/plate with and without S9 | negative | Indicator cells:<br>S. typh.TA 98, TA 100, TA 1535, TA 1537<br>E.coli WP2uvrA | [a]Matsumoto, 2001 |
| chromosome aberration (OECD 473)<br>Short-term treatment: 6.25-50 µg/ml without S9, 25-200 µg/ml with S9.<br>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)<br>Short-term treatment: 0-80 µg/ml without S9, 0-320 µg/ml with S9.<br>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)<br>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)<br>0, 500, 1000 and 2000 mg/kg bw | negative | Mouse bone marrow | [a]Matsumoto, 2003b |
| UDS *in vivo* (OECD 486) | negative | Rat hepatocytes | [a]Buskens, 2007 |

| | | | |
|---|---|---|---|
| 0, 1000 and 2000 mg/kg bw | | | |

[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 | $WP_2uvrA$ | 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 | 100 | 100 | 2 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 3 | 3 |
| Control | 100 | 100 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 |
| (DMSO) | 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) |
| | 100 | 95 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6.25 | 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) |
| | 100 | 89 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12.5 | 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) |
| | 100 | 71 | 6 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 2 | 2 |
| 25 | 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) |
| | 81 | 39 | 1 | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| 50 | 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 | 100 | 99 | 0 | 2 | 9 | 22 | 3 | 1 | 0 | 0 | 28 | 26 |
| Control | 100 | 100 | 1 | 8 | 16 | 27 | 4 | 0 | 0 | 0 | 42 | 40 |
| (MMC) | 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 | 100 | 100 | 0 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 3 | 2 |
| Control | 100 | 100 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| (DMSO) | 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) |
| | 100 | 96 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25 | 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) |
| | 100 | 92 | 1 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 2 | 2 |
| 50 | 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 | 78 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 |
| 100 | 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) |
| | 100 | 24 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 2 | 2 |
| 200 [b)] | 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 | 100 | 63 | 0 | 10 | 10 | 42 | 0 | 1 | 0 | 0 | 48 | 45 |
| Control | 100 | 64 | 1 | 8 | 17 | 40 | 0 | 0 | 1 | 0 | 55 | 52 |
| (B(a)P) | 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 | 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 | 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 | 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 | 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
  - 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
  - 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
  - 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
  - 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
  - 0; 2.5; 5.0; 10.0; 20.0, 40.0 μg/mL
- 4-hour exposure, 28-hour sampling time, with S9 mix
  - 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 |
| | | 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: number of wells with mutants per selection plate (1) | | | | | | | | | | total number of mutants | | | cloning efficiency (at day 2): n° of empty wells per cloning plate | | total number of empty wells | CE x 100% | mutation frequency per $10^6$ survivors | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | | 2 | | 3 | | 4 | | 5 | | | | | 1 | 2 | | | total | small | large |
| without metabolic activation | | | | | | | | | | | | | | | | | | | | |
| | s | l | s | l | s | l | s | l | s | l | s | l | s+l | | | | | | | |
| 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 $p \leq 0.001$

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<br>3 males/dose/sampling time | **Result** | : | not genotoxic |

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** |
| | Negative control | | | | | |
| 14 | 0 | 0.6 | 1.4 | | 0 | |
| | Positive control | | | | | |
| 16 | 10 | 44.7 | 12.7 | | 100 | |
| | Cyflumetofen | | | | | |
| 18 | 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 = $\sigma_{n-1}$
% cells in repair : repair is taken as NNG ≥ 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 |
| | Negative control | | | | | |
| 4 | 0 | 0.7 | 1.3 | | 0 | |
| | Positive control | | | | | |
| 6 | 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 = $\sigma_{n-1}$
% cells in repair : repair is taken as NNG ≥ 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.

## 4.10 Carcinogenicity

**Table 69 Summary table of relevant carcinogenicity studies**

| Method | Results | Remarks | Reference |
|---|---|---|---|
| Carcinogenicity study (24 months) in rats, Fischer (F344/DuCrj)<br>OECD 451<br>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)<br>NOAEL : 20.3 mg/kg bw/day (f)<br><br>LOAEL: 49.5 mg/kg bw/day (m);<br>LOAEL: 61.9 mg/kg bw/day (f) | Hypertrophy of the adrenal cortex and luminal dilatation of the gland in the uterine horn.<br>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)<br>OECD 451<br>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)<br>NOAEL : 144 mg/kg bw/day (f)<br><br>LOAEL: 537 mg/kg bw/day (m);<br>LOAEL: 483 mg/kg bw/day (f) | Vacuolation of the adrenal cortex .<br>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)<br>OECD 451, EPA 870.4200; JMAFF No 12 Nosan No 8147<br>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);<br>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)<br>OECD 451, JMAFF No 12 Nosan No 8147, EPA 870.4200<br>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);<br>LOAEL: 287 mg/kg bw/day (f) | Increased incidence of Leydig cell tumours.<br>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)<br>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) $10^3$/µL | (L ) % of Control | Eosinophil (E) $10^3$/µL | (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 ≤0.05; ** p ≤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)<br>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 | | | | | | | | | i[a] | | |
| - spleen | | | | | | | | | 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)<br>< 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)<br>< 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.

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) $10^3$/µL** | **(L ) % of Control** | **Eosinophil (Eo) $10^3$/µL** | **(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:<br>[N]<br>Soiled fur, external genital | <br>[41]<br>1 | <br>[43]<br>1 | <br>[39]<br>2 | <br>[38]<br>11** |
| Testis:<br>[N]<br>Atrophy<br>Mass(es)<br>Softening | <br>[41]<br>7<br>31<br>16 | <br>[43]<br>1*<br>41**<br>2** | | |
| Epididymis:<br>[N]<br>Atrophy | <br>[41]<br>34 | <br>[43]<br>42* | | |
| Animals killed in extremis or found dead | | | | |
| Systemic/external appearance:<br>[N]<br>Soiled fur, external genital | <br>[9]<br>4 | <br>[7]<br>1 | <br>[11]<br>4 | <br>12]<br>6 |
| Testis:<br>[N]<br>Atrophy<br>Mass(es)<br>Softening | <br>[9]<br>5<br>3<br>3 | <br>[7]<br>0*<br>7*<br>0 | | |
| Epididymis:<br>[N]<br>Atrophy | <br>[9]<br>6 | <br>[7]<br>6 | | |
| All animals examined | | | | |

| Organs and lesions | Sex and dose level (ppm) | | | |
|---|---|---|---|---|
| | MALE | | FEMALE | |
| | 0 | 6000 | 0 | 6000 |
| Systemic/external appearance:<br>[N]<br>Soiled fur, external genital | <br>[50]<br>5 | <br>[50]<br>2 | <br>[50]<br>6 | <br>[50]<br>17** |
| Testis:<br>[N]<br>Atrophy<br>Mass(es)<br>Softening | <br>[50]<br>12<br>34<br>19 | <br>[50]<br>1**<br>48**<br>⇓2** | | |
| Epididymis:<br>[N]<br>Atrophy | <br>[50]<br>40 | <br>[50]<br>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:<br>Atrophy, acinar cell, focal | <br>15/50 | <br>27/50* | <br>5/50 | <br>2/50 |
| Thyroid: | | | | |
| Hyperplasia, C-Cell | 17/50 | 18/50 | 16/49 | 14/50 |
| Adrenal:<br>Hypertrophy, cortical cell, diffuse | <br>7/50 | <br>44/50** | <br>5/50 | <br>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:<br>Tumour, interstitial cell | [41]<br>33 | [43]<br>↑41 | | |
| Thyroid gland:<br>Adenoma, C-cell<br>Carcinoma, C-cell<br>Adenoma/carcinoma, C-cell | [41]<br>11<br>7<br>17 | [43]<br>14<br>13<br>25 | [39]<br>7<br>2<br>9 | [38]<br>4<br>2<br>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:<br>Tumour, interstitial cell | [9]<br>5 | [7]<br>7 | | |
| Thyroid gland:<br>Adenoma, C-cell<br>Carcinoma, C-cell<br>Combined adenoma/carcinoma, C-cell | [9]<br>0<br>2<br>2 | [6]<br>1<br>2<br>3 | [10]<br>2<br>0<br>2 | [12]<br>1<br>2<br>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: | [50] | [50] | | |

| Organs and lesions | Sex and dose level (ppm) | | | |
|---|---|---|---|---|
| | MALE | | FEMALE | |
| | 0 | 6000 | 0 | 6000 |
| Tumour, interstitial cell | 38 | ⇑48 | | |
| Thyroid gland:<br>Adenoma, C-cell<br>Carcinoma, C-cell<br>Adenoma/carcinoma, C-cell | [50]<br>11<br>9<br>19 | [49]<br>15<br>15<br>↑28 | [49]<br>9<br>2<br>11 | [50]<br>5<br>4<br>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<br>86% | 42/50<br>84% | 43/50<br>86% | 49/50<br>98% | 39/50<br>78% | 35/50<br>70% | 43/50<br>86% | 42/50<br>84% | 36/50<br>72% | 31/50<br>62% | 28/50<br>56% | 36/50<br>72% |
| Study 2 | 42/50<br>84% | 48/50<br>96% | 42/50<br>84% | 48/50<br>96% | 40/50<br>80% | | 43/50<br>86% | 41/50<br>82% | | | | |
| Study 3 | 44/50<br>88% | 46/50<br>92% | | | 39/50<br>78% | | | | | | | |
| Study 4 | | 50/50<br>100% | | | 34/50<br>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 |
|---|---|---|---|---|---|---|

| 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≤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 | | 8 | 8 | 20 | 13 | 8 | 14 | 12 | 9 |
| (%) | | (16) | (16) | (40) | (26) | (16) | (28) | (24) | (18) |
| Carcinoma, C-cell | | 0 | 0 | 2 | 4 | 0 | 4 | 2 | 5 |
| (%) | | (0) | (0) | (4) | (8) | (0) | (8) | (4) | (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 | | 9 | 16 | 20 | 21 | 22 | 10 | 14 | 18 | 11 |
| (%) | | (18) | (32) | (40) | (42) | (44) | (20) | (28) | (36) | (22) |
| Carcinoma, C-cell | | 6 | 3 | 2 | 7 | 3 | 5 | 9 | 7 | 9 |
| (%) | | (12) | (6) | (4) | (14) | (6) | (10) | (18) | (14) | (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 | | | | | | | | |
| Carcinoma, C-cell | 3 | 4 | 1 | 1 | 3 | 1 | 4 | 0 |
| Adenoma/carcinoma, C-cell | 0 | 0 | 2 | 0 | 0 | 1 | 4 | 2 |
| | 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 Carc. 2 (H351: Suspected of causing cancer) is therefore proposed.

### 4.10.6 Conclusions on classification and labelling

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

## 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)<br><br>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<br><br>NOAEL developmental: 10 mg/kg bw/d<br><br>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<br><br>LOAEL developmental: 34.6 mg/kg bw/day based on increased adrenal weight and hypertrophy of adrenal cortical cells<br><br>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)<br><br>0, 50, 250 or 1000 mg/kg bw/d | NOAEL parental: 50 mg/kg bw per day<br><br>NOAEL developmental: 50 mg/kg bw/d<br><br>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<br><br>LOAEL developmental: 250 mg/kg bw/day based on delayed ossification<br><br>Teratogenicity: No findings | [a]York, 2001 |
| Teratogenicity study rabbit, Hra:(NZW) (oral gavage)<br><br>0, 50, 250 or 1000 mg/kg bw/d | NOAEL parental: 50 mg/kg bw per day<br><br>NOAEL developmental: 250 mg/kg bw/d<br><br>NOAEL teratogenicity: ≥ 1000 mg/kg bw/d | LOAEL maternal: 250 mg/kg bw/day based on decreased body weight gain<br><br>LOAEL developmental: 1000 mg/kg bw/day based on incomplete ossification, hyoid changes and reduced foetal weight.<br><br>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<br>F1: during weaning, premating period until sacrifice<br>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** | |
| **$F_0$ 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 | | | ica,r | | | ica,r | ica,r | ica,r | f |
| - pituitary | | | | | | | icr | ica,r | |
| - ovaries | | | | | | | | icr | |
| - thyroids | | | | | | | | ia, icr | |
| - liver | | | | | | | | | |
| **Pathology** | | | | | | | | | |
| Macroscopy | | | | | | | | | |
| - adrenal gland: white colour | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 1/24 | 5/24 | 4/24 | |
| - adrenal gland: enlarged | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 1/24 | 0/24 | 5/24 | |
| Microscopy | | | | | | | | | |
| adrenal: | | | | | | | | | |
| -hypertrophy, zona glomerulosa | 4/24 | 4/24 | 4/24 | 4/24 | 4/24 | 13/24 | 14/24 | 19/24 | f |
| -hypertrophy, zona fasciculata | 0/24 | 6/24 | 0/24 | 7/24 | 0/24 | 10/24 | 0/24 | 18/24 | |
| -vacuolation, zona fasciculata | 1/24 | 0/24 | 6/24 | 0/24 | 9/24 | 0/24 | 4/24 | 0/24 | |
| **$F_1$ 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 | | | | | | | | |

| **Dose (mg/kg food)** | **0** | | **150** | | **500** | | **1500** | | **dr** |
|---|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | |
| **Body weight** | No treatment-related findings | | | | | | | | |
| **Organ weights** | | | | | | | | | |
| - adrenals | | | | | ica,r | ica,r | ica,r | ica,r | m/f |
| **Pathology** | | | | | | | | | |
| Macroscopy | No treatment-related findings | | | | | | | | |
| Microscopy | | | | | | | | | |
| adrenal: | | | | | | | | | |
| -hypertrophy, zona glomerulosa | 0/23 | 0/23 | 1/22 | 1/22 | 6/23 | 3/23 | 11/23 | 13/23 | m |
| -hypertrophy, zona fasciculata | 5/23 | 3/23 | 2/22 | 3/22 | 8/23 | 7/23 | 21/23 | 17/23 | 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 | | | | | | ica,r | icr | ica,r | |
| - prostate | | | | | | | dca | | |
| - pituitary | | | | ica | | | | icr | |
| - uterus | | | | ica | | | | | |
| **Pathology** | | | | | | | | | |
| Macroscopy | | | | | | | | | |
| - adrenal: white colour | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 2/24 | 9/24 | 15/24 | |
| - adrenal: enlarged | 0/24 | 0/24 | 0/24 | 0/24 | 0/24 | 1/24 | 0/24 | 9/24 | |
| Microscopy | | | | | | | | | |
| adrenal: | | | | | | | | | |
| -hypertrophy, zona glomerulosa | 4/24 | 1/24 | 5/24 | 5/24 | 9/24 | 7/24 | 11/24 | 21/24 | m/f |
| -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 | |
| 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** | | | | | | | | | |

| **Dose (mg/kg food)** | **0** | | **150** | | **500** | | **1500** | | **dr** |
|---|---|---|---|---|---|---|---|---|---|
| | **m** | **f** | **m** | **f** | **m** | **f** | **m** | **f** | |
| - FSH<br>- progesterone<br>- 17β-oestradiol | | | | dc | | dc<br>dc | | dc<br>dc<br>dc | f |
| **<u>$F_2$ pups</u>** | | | | | | | | | |
| **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**<br>day 7-21 post-partum | | | | | | | dc | dc | |
| **Anogenital distance** | | | icr | | | | icr | | |
| **Organ weights**<br>- adrenals<br>- spleen<br>- thymus<br>- brain | | | | <br><br>dca<br> | ica,r | ia, icr<br><br>dca,r<br> | ica,r<br><br><br>icr | ica,r<br>dca<br>dca<br>icr | m/f |
| **Pathology** | | | | | | | | | |
| <u>Macroscopy</u> | No treatment-related findings | | | | | | | | |
| <u>Microscopy</u><br>adrenal:<br>-hypertrophy, zona glomerulosa<br>-hypertrophy, zona fasciculata | <br><br>3/23<br>1/23 | <br><br>2/23<br>4/23 | <br><br>2/23<br>0/23 | <br><br>3/23<br>4/23 | <br><br>6/21<br>8/21 | <br><br>0/21<br>4/21 | <br><br>14/24<br>22/24 | <br><br>10/23<br>16/23 | <br><br><br>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 |

* $p \leq 0.05$; ** $p \leq 0.01$ (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 ± S.D.) | |
|---|---|---|---|
| | | Parental (F0) generation | F1 generation |
| 1 | 2004 | 4.1 ± 0.3 | 4.2 ± 0.3 |
| 2 | 2005 | 4.0 ± 0.1 | 4.2 ± 0.4 |
| 3 | 2005 | 4.0 ± 0.2 | 4.1 ± 0.3 |
| 4 | 2007 | 4.1 ± 0.2 | 4.0 ± 0.1 |
| 5 | 2007 | 4.0 ± 0.2 | 4.3 ± 0.5 |
| 6 | 2008 | 4.1 ± 0.2 | 4.3 ± 0.5 |
| 7 | 2008 | 4.1 ± 0.4 | 4.1 ± 0.2 |
| 8 | 2009 | 4.0 ± 0.2 | 4.0 ± 0.1 |
| 9 | 2009 | 4.0 ± 0.2 | 4.2 ± 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 ± S.D.) | |
|---|---|---|---|
| | | Days of age | Body weights (g) at completion |
| 1 | 2004 | 41.3 ± 2.0 | 176 ± 16 |
| 2 | 2005 | 40.7 ± 1.5 | 179 ± 16 |
| 3 | 2005 | 42.3 ± 2.0 | 182 ± 10 |
| 4 | 2007 | 41.2 ± 1.8 | 175 ± 14 |
| 5 | 2007 | 41.0 ± 1.4 | 184 ± 14 |
| 6 | 2008 | 42.3 ± 1.4 | 188 ± 13 |
| 7 | 2008 | 42.3 ± 2.4 | 182 ± 19 |
| 8 | 2009 | 42.3 ± 1.9 | 181 ± 10 |
| 9 | 2009 | 42.5 ± 2.1 | 189 ± 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 ± S.D.) | |
|---|---|---|---|
| | | Days of age | Body weights (g) at completion |
| 1 | 2004 | 31.8 ± 2.0 | 102 ± 10 |
| 2 | 2005 | 30.8 ± 2.4 | 97 ± 12 |
| 3 | 2005 | 31.0 ± 2.3 | 97 ± 11 |
| 4 | 2007 | 31.6 ± 1.8 | 97 ± 10 |
| 5 | 2007 | 28.2 ± 1.3 | 84 ± 7 |
| 6 | 2008 | 30.3 ± 1.5 | 92 ± 10 |
| 7 | 2008 | 31.9 ± 2.4 | 101 ± 11 |
| 8 | 2009 | 29.7 ± 2.5 | 90 ± 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<br><br>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)<br>< 319 (females) | 250 (males)<br>319 (females) | Hyperplasia of the interstitial cells of the testes.<br>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 | | | ic[a,r] | ic[a,r] | f |
| - adrenal right | | | | ic[a,r] | |
| **Pathology** | | | | | |
| macroscopy | No treatment-related findings | | | | |
| microscopy | | | | | |
| adrenal cortical cells: | | | | | |
| -diffuse hypertrophy | 0/25 | 0/25 | 0/25 | 14/25** | |
| -diffuse bilateral cytoplasmic vacuolation | 0/25 | 0/25 | 2/25 | 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 | 32 | 14 | 12 | 17 | |
| - late | 0 | 1 | 0 | 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 ± 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 ≥ 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**<br>- day 6-29 | | | d | d | |
| **Food consumption**<br>day 18-21 of gestation | | | d | dc | |
| **Gravid uterine weight** | | | d | d | |
| **Organ weights**<br>- 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**<br>- early<br>- late | <br>0.2<br>0.7 | <br>0.2<br>0.1 | <br>0.1<br>0.2 | <br>0.1<br>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.

### 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)<br>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<br>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.<br>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)<br>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)<br>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,<br>Beagle<br>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);<br>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 | | | | | | $ic^{a}$, $i^{r}$ | |
| **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<br><br>(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.<br><br>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))<br><br>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<br><br>US EPA OPPTS 890.1550 | Estradiol induction:<br><br>NOEC 1 µM; LOEC 5 µM<br><br>Testosterone inhibition:<br><br>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.<br><br>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)<br><br>US EPA OPPTS 890.1250 | Negative in the tested range of $1x10^{-10}$ – $1x10^{-4}$ M | This assay is part of the Tier 1 screening battery of the Endocrine Disruptor Screening Program (EDSP) of the US EPA<br><br>Cyflumetofen is classified as 'not interactive' for ER binding | [a]Akhurst, L 2011 |
| Androgen Receptor Binding (Rat Prostate Cytosol)<br><br>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.<br><br>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 of1 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 × 10-10 to 1 × 10-4 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.

# 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

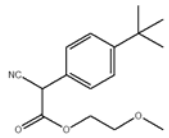
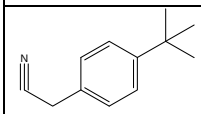
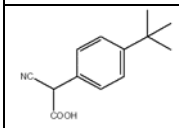
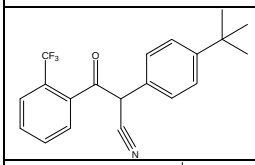
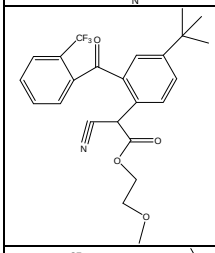
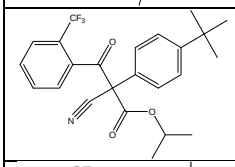
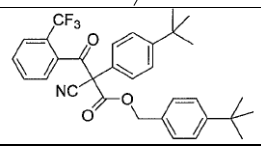
**Table 123 Summary of relevant information on degradation**

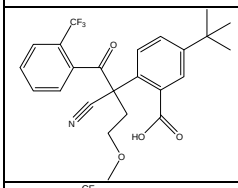
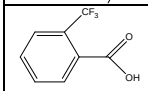
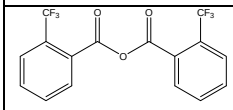
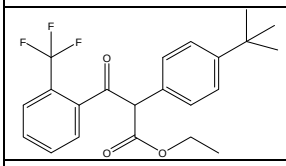
| Method | Results | Remarks | Reference |
|---|---|---|---|
| Hydrolysis<br>(JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993)) | $DT_{50}$ pH 4 = 7.7 days (25°C)<br>$DT_{50}$ pH 5 = 6.0 days (25°C)<br>$DT_{50}$ pH 7 = 9.8 hours (25°C)<br>$DT_{50}$ pH 9 = 10.3 min (25°C) | Metabolites > 10 %: A-1, A-2, A-18, B-1, AB-1 | Nakamura, 2004g (IIA7.5/01) [a] |
| Photolysis, natural and sterilized water<br>(JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993)) | $DT_{50}$ natural water: 1.07 hr (25°C)<br>$DT_{50}$ at pH5: 1.28 hr (25°C)<br>Quantum yield $3.0x10^{-5}$ mole/Einstein | Specific photolytic degradation products > 10 %: AB-7 and AB-15<br>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<br>(OECD 308) | Cyflumetofen:<br>$DT_{50}$ water = 0.1 days ($SW^b$ system) and 0.7 days ($GV^c$ system)<br>$DT_{50}$ sediment = 2.1 days ($SW^b$ system) and 12 days ($GV^c$ system)<br>$DT_{50}$ system = 0.2 days ($SW^b$ system) and 9.9 days ($GV^c$ system)Metabolite A-2:<br>$DT_{50}$ system: 11.74 days ($GV^c$ system)<br>Metabolite AB-1:<br>$DT_{50}$ system: 18.29 days ($GV^c$ system)<br>Metabolite Met8<br>$DT_{50}$ system: 3.09 days ($SW^b$ system) | A-ring $^{14}C$ labelled cyflumetofen; results of 2 water/sediment systems.<br>Relevant (> 10%) metabolites: Met1, A-2, Met8, AB-11. | Noorloos and Brands, 2008 (IIA 7.8.3-01) [a] |
| Water/sediment system, aerobic<br>(OECD 308) | Cyflumetofen:<br>$DT_{50}$ water = 0.05 days ($SW^b$ system) and 0.1 days ($GV^c$ system)<br>$DT_{50}$ sediment = 0.6 days ($SW^b$ system) and 15 days ($GV^c$ system)<br>$DT_{50}$ system = 0.08 days ($SW^b$ system) and 14 days ($GV^c$ system)<br>Metabolite B-1:<br>$DT_{50}$ system = 320 days ($GV^c$ system)<br>Metabolite B-2:<br>$DT_{50}$ 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.<br>Relevant (> 10%) metabolites: B-1, B-2. | Noorloos and de Mol, 2007 (IIA 7.8.3-02) [a] |
| Photochemical oxidative degradation in air<br>(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 $1.5x10^6$ 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)<br><br>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)<br><br>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)<br><br>Sediment: 21.5% | 190.1 | 2.49 | 196.03 (267.4, ECOSAR v0.99h) | 0.3986 |
|  | B-2 | Water: 15.4% (B)<br><br>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]<br><br>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.<br>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-$^{14}$C]Cyflumetofen (batch CFQ12736)

Portions of 2.5 mL [A-ring-U-$^{14}$C]Cyflumetofen or [B-ring-U-$^{14}$C]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-$^{14}$C]Cyflumetofen (all pH values) and between 97.62 and 102.15% AR for [B-ring-U-$^{14}$C]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.

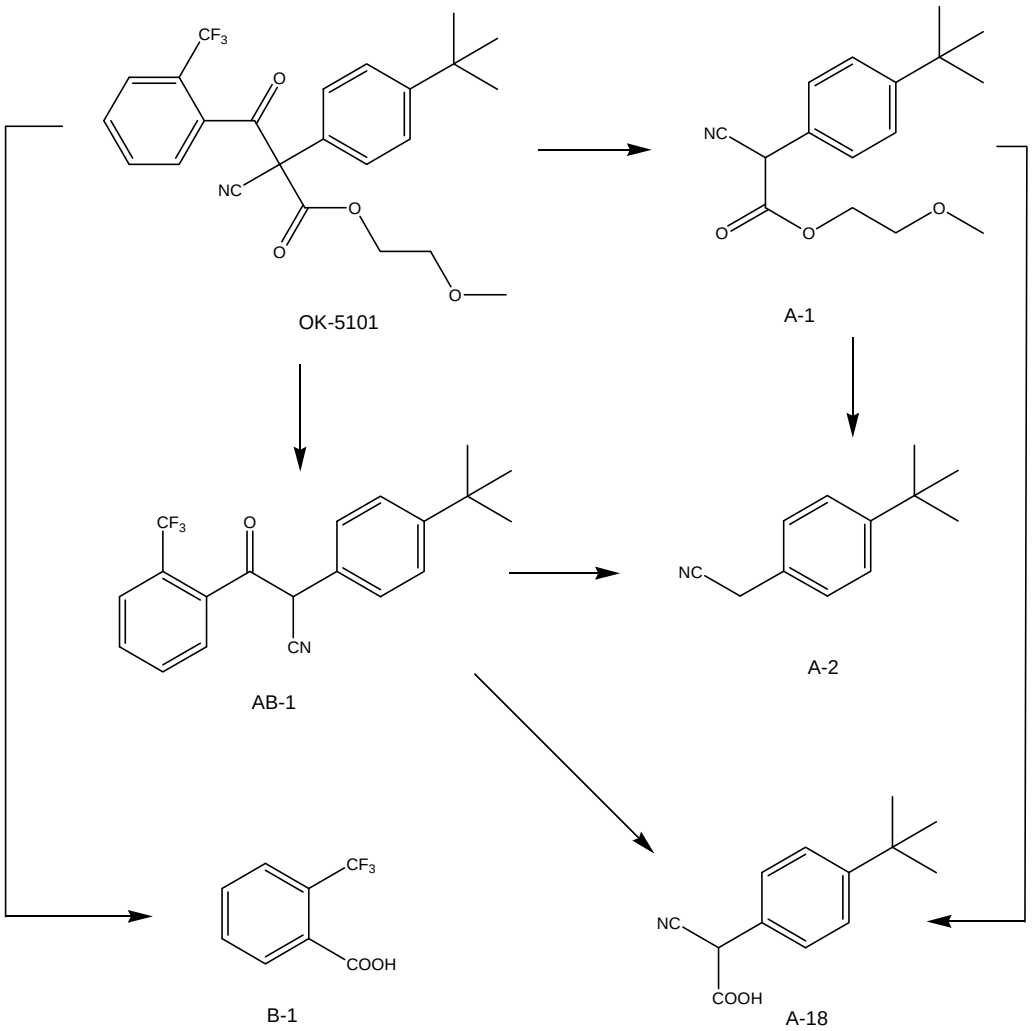



Figure 2 Hydrolysis pathway of cyflumetofen (in above figure indicated as OK-5101)

*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)<br>[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.

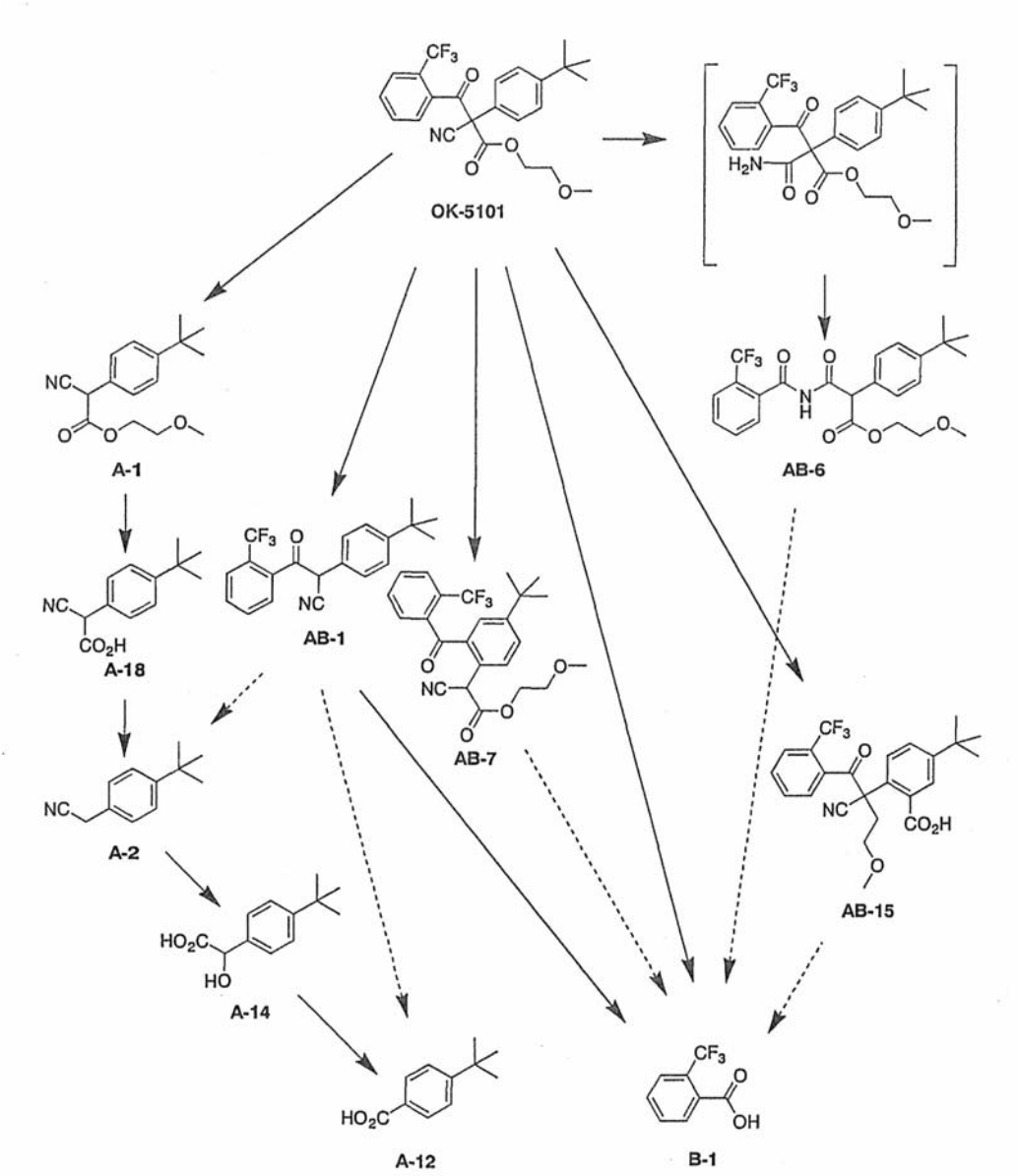



**Figure 3 Aquatic photolysis pathway of cyflumetofen (indicated as OK-5101 in the figure)**

*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 $9.7 \times 10^5$ molecules $cm^{-3}$ (Atkinson calculation). This was subsequently recalculated by the Rapporteur Member State based on $1.5 \times 10^6$ 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).

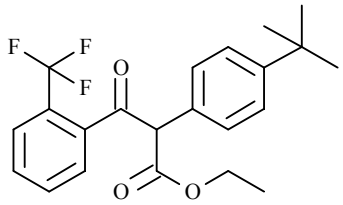
Only very small amounts of radioactivity (≤0.7% of applied) were found in the polyurethane foam plugs and the ethylene glycol monoethyl ether traps. Approximately 20% of applied was recovered as $CO_2$ at the end of the test.

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 | $\chi^2$ (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 | $\chi^2$ (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 ($day^{-1}$) | $\chi^2$ (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).

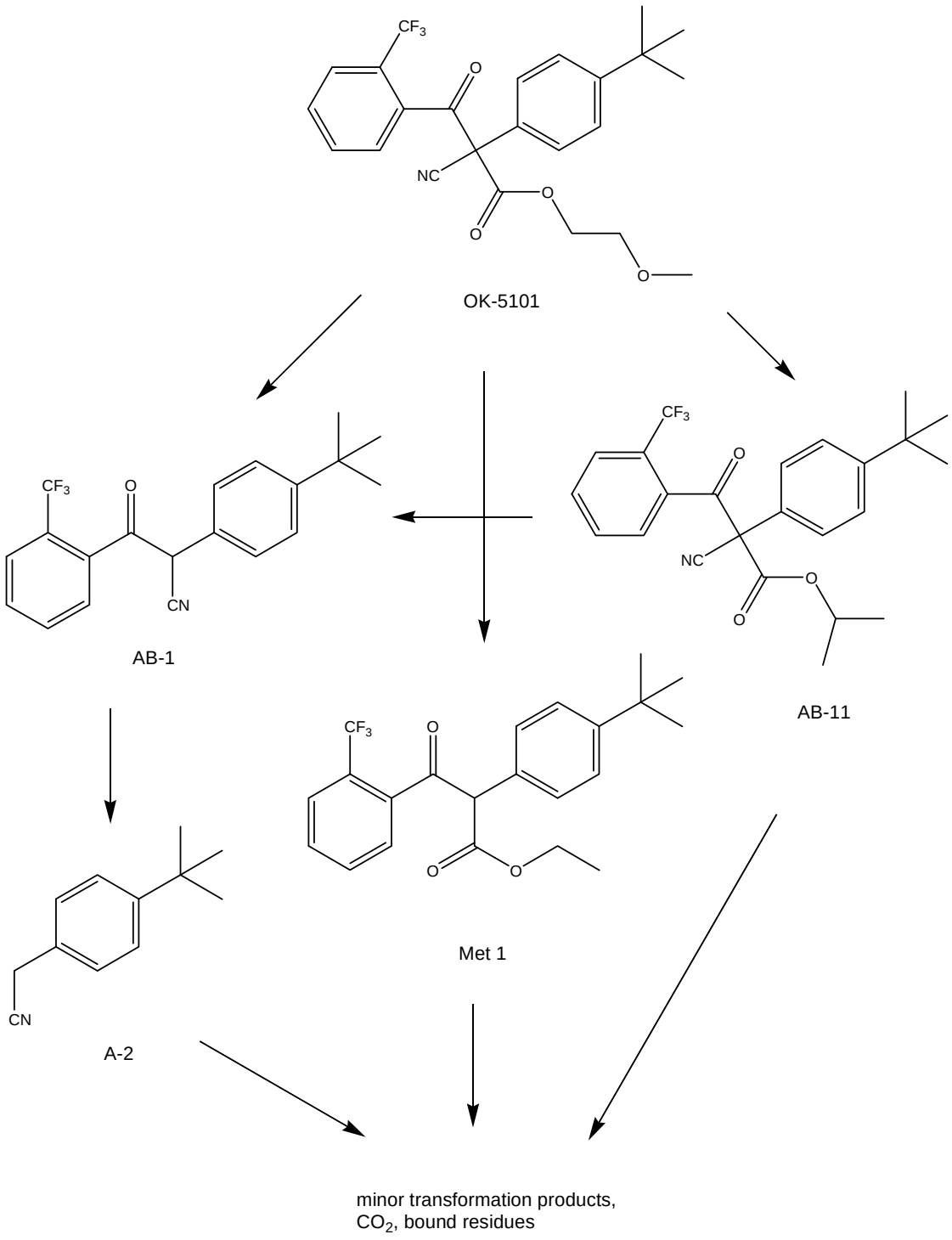



**Figure 4 Degradation pathway of cyflumetofen (indicated as OK-5101) in water-sediment (A-label)**

*$^{14}C$ 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<br>14.1-15.9 min | Met-2[1]<br>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 | $\chi^2$ (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 | $\chi^2$ (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 ($day^{-1}$) | $\chi^2$ (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).

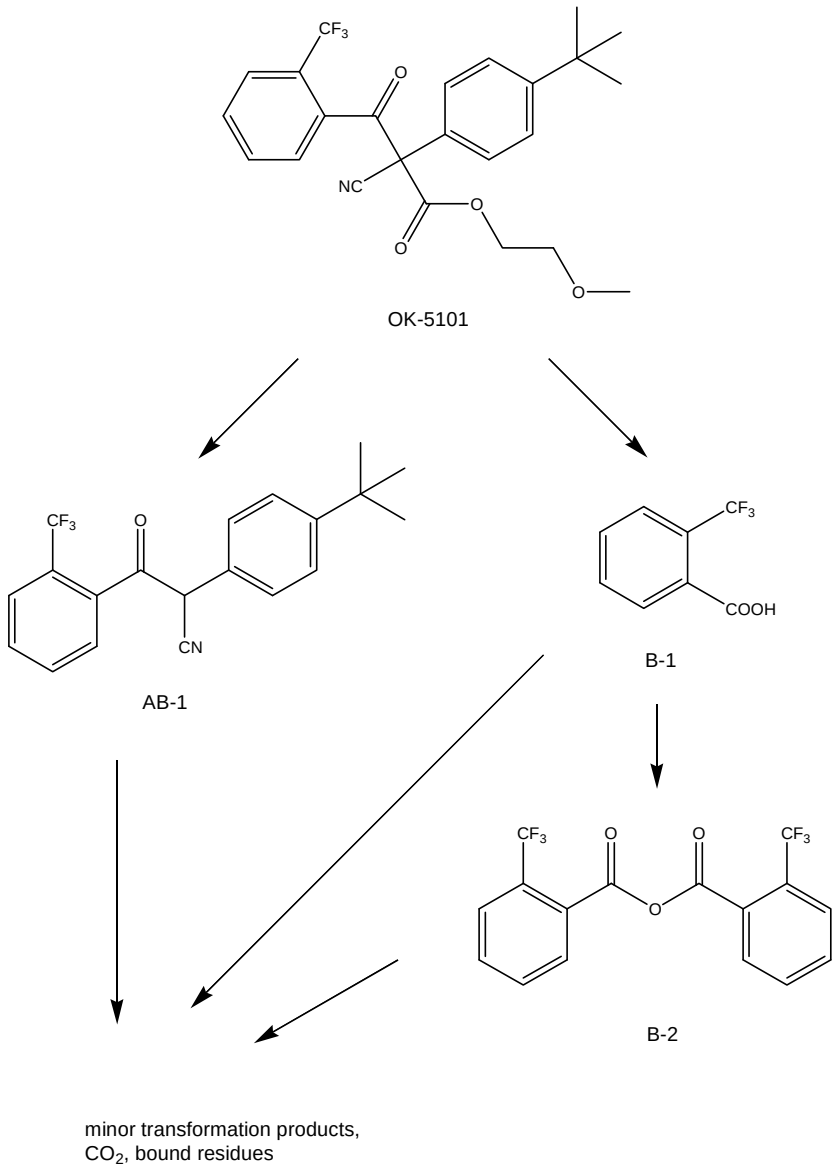



**Figure 5** Degradation pathway of cyflumetofen (indicated as OK-5101) in water-sediment (B-label)

### 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 ($DT_{50s}$ < 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 (<$5.9x10^{-6}$ Pa at 25°C) and a medium calculated Henry's law constant of < $9.4x10^{-2}$ $Pa.m^3.mol^{-1}$ 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)<br>(OECD 305; EC C.13) | 170 (for total radioactivity)<br>< 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.

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.

[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 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**

| | 1.0 µg/L nominal | | 10 µg/L nominal | |
|---|---|---|---|---|
| **Parameter** | **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: $NOEC_{reproduction}$ ≥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 20°C 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 (≥ 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".

<u>CLP- Acute aquatic hazards</u>

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 ≤ 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.

<u>CLP – Long-term aquatic hazards</u>

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.

# 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

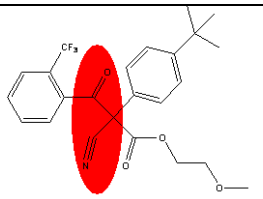
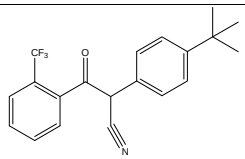
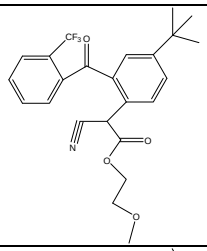
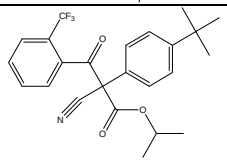
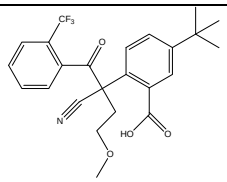
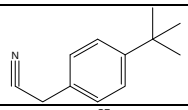
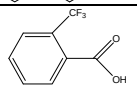
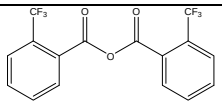
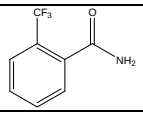
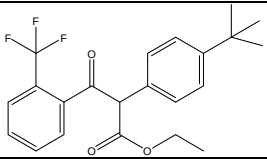
A reference list for all individual studies and references is added separately in a confidential document and will be included in the IUCLID file.

# 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)<br><br>presumed active site is indicated by shaded area | | 447.5 | 4.3(A)<br>4.69(B)<br>6.18(C) |
|  | AB-1 | Sediment: 15<br>Soil: 22 | 345.4 | 5.33(B)<br>5.46(C) |
|  | AB-7 | Water: 11<br>(photolysis) | 447.5 | 4.91(B)<br>5.00(C) |
|  | AB-11 | Water: 10<br>Sediment: 10 | 431.5 | 5.87(B)<br>7.32(C) |
|  | AB-15 | Water: 55<br>(photolysis) | 447.5 | 5.05(B)<br>5.87(C) |
|  | A-2 | Water: 18 | 173.3 | 3.47(B)<br>3.14(C) |
|  | B-1 | Water: 65<br>Sediment: 22<br>Soil: 63<br>Plant leaves: 9.1 | 190.1 | 2.49(B)<br>2.87(C) |
|  | B-2 | Water: 15<br>Sediment: 28 | 362.2 | 4.26(B)<br>4.96(C) |
|  | B-3 | Soil: 23 | 189.1 | 0.97(B)<br>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