EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

EC Number: 405-060-5

CAS Number: 118712-89-3

CLH-O-0000006955-61-01/F

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

Adopted

18 March 2021

CLH report

Proposal for Harmonised Classification and Labelling

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

International Chemical Identification:

Transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

EC Number: **405-060-5**

CAS Number: **118712-89-3**

Index Number: **607-223-00-8**

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Version number: 2.0 **Date: October 2019**

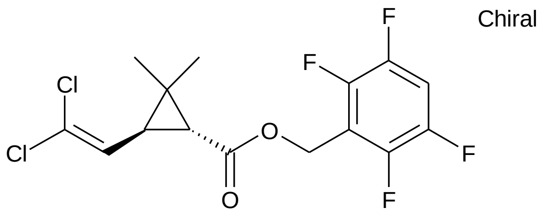
ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON TRANSFLUTHRIN (ISO); 2,3,5,6-TETRAFLUOROBENZYL (1R,3S)-3-(2,2-DICHLOROVINYL)-2,2-DIMETHYLCYCLOPROPANECARBOXYLATE

CONTENTS

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 1: Substance identity and information related to molecular and structural formula of the substance

Name(s) in the IUPAC nomenclature or other international chemical name(s)	2,3,5,6-tetrafluorobenzyl (1R,3S)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate; 2,3,5,6-tetrafluorobenzyl (1R)-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate;
Other names (usual name, trade name, abbreviation)	Cyclopropanecarboxylic acid, 3-(2,2-dichloroethenyl)-2,2-dimethyl-, (2,3,5,6-tetrafluorophenyl) methyl ester, (1R, 3S);
ISO common name (if available and appropriate)	Transfluthrin
EC number (if available and appropriate)	405-060-5 *
EC name (if available and appropriate)	2,3,5,6-Tetrafluorobenzyl trans-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropanecarboxylate *
CAS number (if available)	118712-89-3 *
Other identity code (if available)	CIPAC No: 741 Index no: 607-223-00-8 *
Molecular formula	$C_{15}H_{12}Cl_2F_4O_2$
Structural formula	
SMILES notation (if available)	Fc1c(F)cc(F)c(F)c1COC(=O)C2C(C)(C)C2C=C(Cl)Cl
Molecular weight or molecular weight range	371.2 g/mol
Information on optical activity and typical ratio of (stereo) isomers (if applicable and appropriate)	1R, trans-configuration. The cis/trans, S-isomers and 1R,cis-isomer are considered impurities.
Description of the manufacturing process and identity of the source (for UVCB substances only)	Not applicable.
Degree of purity (%) (if relevant for the entry in Annex VI)	Transfluthrin (ISO) is produced at a minimum purity of 96.5%, referring to a 1R, trans-configuration. The cis/trans, S-isomers and 1R,cis-isomer are considered impurities.

** The EU index no. and EC no. refer to the 1R,trans and 1S,trans configurations, which is not in agreement with the definition of transfluthrin (ISO), which is exclusively the 1R,trans isomer. The CAS registry no. does refer to the correct isomer.*

1.2 Composition of the substance

Table 2: Constituents (non-confidential information)

Constituent (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum in multi-constituent substances)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**
Transfluthrin (ISO)	96.5-100%	Skin Irrit. 2, H315 Aquatic Acute 1, H400 Aquatic Chronic 1, H410	Skin Irrit. 2, H315 Aquatic Acute 1, H400 Aquatic Chronic 1, H410 M = 1000 M (chronic) = 1000 (by 1 of 21 notifiers, accessed 17-10-2017)

Table 3: Impurities (non-confidential information) if relevant for the classification of the substance

Impurity (Name and numerical identifier)	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The impurity contributes to the classification and labelling**
no relevant impurities or additives present				

Table 4: Additives (non-confidential information) if relevant for the classification of the substance

Additive (Name and numerical identifier)	**Function**	**Concentration range (% w/w minimum and maximum)**	**Current CLH in Annex VI Table 3.1 (CLP)**	**Current self-classification and labelling (CLP)**	**The additive contributes to the classification and labelling**
no relevant impurities or additives present					

2 PROPOSED HARMONISED CLASSIFICATION AND LABELLING

2.1 Proposed harmonised classification and labelling according to the CLP criteria

Table 5:

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors, ATE	Notes
					Hazard Class and Category Code(s)	**Hazard statement Code(s)**	**Pictogram, Signal Word Code(s)**	**Hazard statement Code(s)**	**Suppl. Hazard statement Code(s)**		
Current Annex VI entry	607-223-00-8	transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl *trans*-2-(2,2-dichlorovinyl)-3,3-dimethylcyclopropane carboxylate	405-060-5	118712-89-3	Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H315 H400 H410	GHS07 GHS09 Wng	H315 H410	-	-	-
Dossier submitters proposal	607-223-00-8	transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate	405-060-5	118712-89-3	**Retain** Aquatic Acute 1 Aquatic Chronic 1 **Add** Acute Tox. 4 Carc. 2 STOT SE 1 STOT RE 2 **Remove** Skin Irrit. 2	**Retain** H400 H410 **Add** H302 H351 H370 (nervous system) H373 (kidney) **Remove** H315	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H302 H351 H370 (nervous system) H373 (kidney) **Remove** H315	**Add** EUH066	**Add** oral: ATE = 583 mg/kg bw M = 1000 M = 1000	-
Resulting Annex VI entry if agreed by RAC and COM	607-223-00-8	transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate	405-060-5	118712-89-3	Acute Tox. 4 Carc. 2 STOT SE 1 STOT RE 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H351 H370 (nervous system) H373 (kidney) H400 H410	GHS07 GHS08 GHS09 Wng	H302 H351 H370 (nervous system) H373 (kidney) H410	EUH066	oral: ATE = 583 mg/kg bw M = 1000 M = 1000	-

Table 6: Reason for not proposing harmonised classification and status under public consultation

Hazard class	Reason for no classification	Within the scope of public consultation
Explosives	Hazard class not assessed in this dossier	No
Flammable gases (including chemically unstable gases)	Hazard class not assessed in this dossier	No
Oxidising gases	Hazard class not assessed in this dossier	No
Gases under pressure	Hazard class not assessed in this dossier	No
Flammable liquids	Hazard class not assessed in this dossier	No
Flammable solids	Hazard class not assessed in this dossier	No
Self-reactive substances	Hazard class not assessed in this dossier	No
Pyrophoric liquids	Hazard class not assessed in this dossier	No
Pyrophoric solids	Hazard class not assessed in this dossier	No
Self-heating substances	Hazard class not assessed in this dossier	No
Substances which in contact with water emit flammable gases	Hazard class not assessed in this dossier	No
Oxidising liquids	Hazard class not assessed in this dossier	No
Oxidising solids	Hazard class not assessed in this dossier	No
Organic peroxides	Hazard class not assessed in this dossier	No
Corrosive to metals	Hazard class not assessed in this dossier	No
Acute toxicity via oral route	Harmonised classification proposed.	Yes
Acute toxicity via dermal route	Data conclusive but not sufficient for classification.	No
Acute toxicity via inhalation route	Data conclusive but not sufficient for classification.	No
Skin corrosion/irritation	Data conclusive but not sufficient for classification.	Yes
Serious eye damage/eye irritation	Hazard class not assessed in this dossier	No
Respiratory sensitisation	Data lacking	No
Skin sensitisation	Hazard class not assessed in this dossier	No
Germ cell mutagenicity	Hazard class not assessed in this dossier	No
Carcinogenicity	Harmonised classification proposed	Yes
Reproductive toxicity	Hazard class not assessed in this dossier	No
Specific target organ toxicity-single exposure	Harmonised classification proposed	Yes
Specific target organ toxicity-repeated exposure	Harmonised classification proposed	Yes
Aspiration hazard	Data lacking	No
Hazardous to the aquatic environment	Harmonised classification proposed	Yes
Hazardous to the ozone layer	Hazard class not assessed in this dossier	No

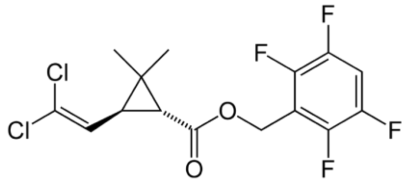
3 HISTORY OF THE PREVIOUS CLASSIFICATION AND LABELLING

Transfluthrin is currently classified as GHS07; H315 (Skin Irrit. 2) and GHS09; H400 (Very toxic to aquatic life) and H410 (Very toxic to aquatic life with long lasting effects) according to Regulation (EC) No 1272/2008, Annex VI. The current EU index no. and EC no. refer to the 1R,trans and 1S,trans configurations, which is not in agreement with the current definition of transfluthrin (ISO). Initially, transfluthrin (ISO) contained both the 1R,trans and 1S,trans configurations, but during the authorization process within the EU, the definition of transfluthrin was changed and currently it exclusively refers to the 1R,trans isomer. More specific, the 1S,trans isomer is currently considered an impurity. This isomer used to be included in the specification for transfluthrin (former specification was 1RS,trans at 985 g/kg), but according to REACH guidance, the 1S,trans isomer should be regarded an impurity. This means that only the IUPAC name of the substance has changed but not the ISO name and the substance itself as produced and tested.

Transfluthrin is not registered under REACH (October, 2017).

Transfluthrin has been evaluated in accordance with Article 11(2) of Directive 98/8/EC for use in product-type 18, insecticides, acaricides and products to control other arthropods, as defined in Annex V to that Directive, which corresponds to product-type 18 (PT18) as defined in Annex V to Regulation (EU) No 528/2012. Transfluthrin was approved on January 1st 2015 as an existing active substance for use in biocidal products for PT18 under Regulation (EU) No 407/2014. The assessment report and study summaries are available at: http://dissemination.echa.europa.eu/Biocides/factsheet?id=1404-18.

On the basis of a review of the submitted data, the following CLP classification and labelling is proposed: GHS07; H302 (Acute Tox. 4), GHS08; H351 (Carc Cat 2), H370 (STOT SE 1) and H373 (STOT RE 2) and GHS09; H400 (m-factor 1000) and H410 (m-factor 1000) according to Regulation (EC) No 1272/2008, Annex VI. Classification with EUH066, repeated exposure may cause skin dryness or cracking, is proposed. In addition, it is proposed to remove the classification as Skin Irrit. 2 (H315).

RAC general comment
Transfluthrin is intended for non-professional users as a fast-acting pyrethroid insecticide. Proposed concentrations of 1 mg/m³ and 0.4 mg/m² have shown immediate knockdown effects on the target organisms. The CLH report has been prepared based on data submitted by the lead registrant in the assessment report for transfluthrin and additional mechanistic studies performed to clarify the mode of action. Structural formula 

Toxicokinetics

Absorption of transfluthrin and/or its hydrolysis products is rapid and is assumed to be 100% for the oral and inhalation route. The highest levels of transfluthrin in tissues (in total less than 2%) were found in liver and kidney and the lowest levels were found in brain. Transfluthrin is rapidly excreted: up to 90% in urine within 48 h.

The benzylmethylene moiety is predominantly metabolized to tetrafluorobenzoic acid (TFBA) and the glucuronic acid conjugate of tetrafluorobenzyl alcohol. The carboxyl moiety is probably metabolised to dichlorochrysanthemic acid (DCCA). The liver is the main organ responsible for metabolism.

4 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Justification that action is needed at Community level is required.

Reason for a need for action at Community level:

Change in existing entry due to new interpretation/evaluation of existing data

Further detail on need of action at Community level

Transfluthrin is an active substance in PT18. A change of the current classification is proposed to RAC and the endpoints carcinogenicity, irritation and acute oral toxicity and aquatic toxicity are highlighted.

5 IDENTIFIED USES

Transfluthrin is a fast-acting pyrethroid insecticide intended for use by non-professional users, and is approved for product-type 18 (insecticides, acaricides and products to control other arthropods).

The active substance transfluthrin at the proposed concentration of 1 mg/m^3 (mosquito coil and vaporiser) and at 0.4 mg/m^2 paper (mothpaper) has been shown to give an immediate knockdown effect for the target organisms. In these tests the following species have been used: Mosquitoes (*Aedes aegypti*, *Culex quinquefasciatus*), house flies (*Musca domestica*), cockroaches (*Blattella germanica*), and moth (*Tineola bisselliella*).

No known resistance in the target species has been observed to-date for this active substance.

6 DATA SOURCES

This CLH report is compiled based on the data on transfluthrin that was submitted and evaluated in the assessment report for transfluthrin (finalised in the Standing Committee on Biocidal Products at its meeting on 13 March 2014) and additional mechanistic studies performed to clarify the mode of action. The assessment report of transfluthrin contains only relevant studies and studies identified as ‘ non-key studies’ were not included in the assessment report. To provide a complete overview of the available data for transfluthrin, these ‘non-key studies’ are included in this CLH report for the endpoints evaluated.

7 PHYSICOCHEMICAL PROPERTIES

Table 7: Summary of physicochemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
Physical state at 20°C and 101,3 kPa	Pure substance (purity: 99.3% w/w): crystalline, white needles; no characteristic odour Technical (purity 99.1% w/w): off-white needles; toluene-like odour	Bogdoll, B and Eyrich, U (2005)	EPA OPPTS 830.6302, 830.6303, 830.6304 and Directive 94/37/EC (Annex 1; 2.4)
Melting/freezing point	Melting point: 32 ºC Batch No. EATFTJ005, purity 99.1% (w/w)	Smeykal, H (2005)	Differential scanning calorimetry in accordance with OECD Guideline 102
Boiling point	242 ºC at 1033 hPa Batch No. 91031ELB01, purity 98.0% (w/w)	Krohn, J (1991)	Siwoloboff method in accordance with OECD Guideline 103 and EEC A 2
Density	1.3856 g/cm ^{3} at 20ºC 1.3624 g/cm ^{3} at 40ºC Batch No. EATFTJ005, purity 99.1% (w/w)	Rexer, K and Bittner, P (2005),	Flexual resonator method using the density meter DMA 38, based on EPA OPPTS 830.7300, DIN 51757 procedure D, EC A.3, and OECD guideline 109
Vapour pressure	20 ºC: 9x10^{-4} Pa 25 ºC: 2x10^{-3} Pa Batch No. APF11088650, purity 97.8 %	Weber, R and Krohn, J (1995),	Gas saturation method in accordance with OECD Guideline 104.
Surface tension	44.8 ± 3.0 dyne/cm [predicted using Chemsketch v.5.0 (Advanced Chemistry Development Inc)]		A study was not provided due to the water solubility (< 1mg/L).
Water solubility	0.057 ± 0.00294 mg/L (20 °C) Batch APF11088650. Purity, 97.8%	Krohn, J (1995)	Column elution method in accordance with OECD Guidelines No. 105.
Partition coefficient n-octanol/water	log K_{ow} = 5.46 temperature: 20 ºC Batch APF11088650. Purity, 97.8%	Krohn, J (1995).	Shake flask method in accordance with OECD-Guidelines No. 107. Comment: Shake flask only suitable to determine log K_{ow} up to 4. Therefore considered unreliable and assigned Klimisch score of 3.
	log *Kow* = 5.5,	Eyrich, U (2017)	HPLC method as described in

Property	Value	Reference	Comment (e.g. measured or estimated)
	independent of pH temperature: 25 ºC Batch AE 0035474-01-08 Purity, 98.1 %		OECD guideline 117. Reliability score of 1. Study was submitted for CLH procedure after substance approval following the evaluation according to the requirements of Directive 98/8/EC.
Flash point	119.0 ºC under atmospheric conditions (1013.3 hPa). Batch No. EATFTJ005, 99.1% (w/w)	Smeykal, H (2005)	Investigated as stipulated in EC test procedure A 9
Flammability	Auto-ignition temperature 415 ºC Batch No. 816779502, purity: 95.7%	Heitkamp, D (2001)	Investigated as stipulated in DIN 51794 and EC Guideline A 15
Explosive properties	Non-explosive according to mechanical sensitivity (shock and friction) and thermal sensitivity tests Batch No. EATFTJ005, purity 99.1% (w/w)	Smeykal, H (2005)	Investigated as stipulated in test EC A14 and OECD guideline 113
Self-ignition temperature	Auto-ignition temperature 415ºC Batch No. 816779502, purity: 95.7%	Heitkamp, D (2001)	Investigated as stipulated in DIN 51794 and EC Guideline A 15.
Oxidising properties	Non-oxidising according to tests conducted under EC A21. Transfluthrin does not have oxidising potential. Batch No. EATFTJ005, purity 99.1% (w/w)	Smeykal, H (2006)	Investigated as stipulated in EC test procedure A21 for the oxidising properties of liquids.
Granulometry	No data available	-	-
Stability in organic solvents and identity of relevant degradation products	Not applicable	-	-
Dissociation constant	Nor applicable since the chemical structure of transfluthrin does not contain any acidic protons or basic centres; therefore, no dissociation	-	-

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
	in water occurs.		
Viscosity	181.9 mPa·s at 20ºC 35.65 mPa·s at 40ºC Batch No. EATFTJ005, purity 99.1% (w/w)	Rexer, K and Bittner, P (2005)	Based on EPA OPPTS 830.7100, DIN 51562 and OECG guideline 114
Henry's law constant	5.86 Pa.m^3/mol at 20 °C	Assessment report (2014)	calculated

8 EVALUATION OF PHYSICAL HAZARDS

Not evaluated in this dossier.

9 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

Table 8: Summary table of toxicokinetic studies

Method	**Results**	**Remarks**	**Reference**
Basic Toxicokinetics in the Rat (no guideline, but methods used are comparable to EC Method B.36.)	Absorption: rapidly absorbed and metabolized in rats. Excretion: 48 hours after oral dosing 96-98% of the administered activity was excreted in the urine and faeces. Approximately 1-2% remained in tissues. The major route of excretion was the urine (74-88%), which was similar in each gender at all but the highest dose group. The highest dose group excreted a greater proportion, although not the majority, in the faeces. This is thought to be due to decreased absorption and/or saturation of enzymatic detoxification systems. Metabolism: The major metabolites of transfluthrin are tetrafluorobenzoic acid (TFBA) and the glucuronic acid conjugate of tetrafluorobenzyl alcohol. The dose-response information that can be derived from this study is that the highest dose tested, 200 mg/kg, appears capable of saturating the uptake/metabolizing enzyme system in the rat. This is not correlated with clinical signs, and therefore does not represent a LOAEL. All other doses, including single 0.5 mg/kg and 5.0 mg/kg, and multiple 0.5 mg/kg doses (15 doses) did not produce metabolic or excretory effects different from each other. In no case were adverse clinical signs noted.	No plasma levels of radioactivity or time curve for excretion of radiolabel were determined. However, it is obvious that plasma half-life times will be short. In addition, plasma half-lives have been determined in other, non-key studies submitted.	Doc. IIIA Section 6.2
Metabolism in female rats	Metabolism: The principal metabolic reactions of [methylene-14C]Transfluthrin in	-	Section A 6.2/02

Method	Results	Remarks	Reference
(US EPA Health Effects Test Guideline, OPPTS 870.7485; Metabolism and Pharmacokinetics EU Council Directive 91/414/EEC amended by the Commission Directive 94/79/EC PMRA Ref.: DACO 4.5.9 Metabolism/Toxicokinetics in Mammals (Lab. Animal) OECD Guideline for Testing Chemicals No. 417, Toxicokinetics Japanese MAFF Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147)	the female rat were: -ester cleavage of the molecule to form Transfluthrin-tetrafluorobenzylalcohol -conjugation of Transfluthrin-tetrafluorobenzylalcohol with glucuronic acid -further oxidation Transfluthrin-tetrafluorobenzylalcohol to Transfluthrin-tetrafluorobenzoic acid -hydroxylation of a methyl group of the cyclopropane ring followed by glucuronidation to the hydroxymethyl-glucuronide -oxidation of the hydroxymethyl group of cyclopropane ring to the carboxylic acid - oxidative and reductive dehalogenation of the dichlorovinyl side chain Distribution: The detection of Transfluthrin in fat and of significant proportions of metabolites with the uncleaved ester moiety demonstrate that unchanged Transfluthrin is the major part of radioactivity absorbed from the gastrointestinal tract after oral dosing of the test compound. Excretion: With regard to urine, transfluthrin-tetrafluorobenzyl-glucuronide and transfluthrin-tetrafluorobenzoic acid were identified as the only metabolites as well.		

9.1 Short summary and overall relevance of the provided toxicokinetic information on the proposed classification(s)

Oral absorption of transfluthrin and/or its hydrolysis products is rapid, and is assumed to be 100%. For the inhalation route 100% absorption is assumed. Route to route extrapolation for effects exerted by intact transfluthrin is not possible. Dermal absorption of transfluthrin is assumed to be 10%, on the basis of a MW of 371 and log Kow of 5.5, and data from other pyrethroids in other formulations. Highest levels of transfluthrin in tissues (in total less than 2%) are found in liver and kidney, lowest levels are found in brain. Excretion is rapid; 74-90% in urine within 48h. There is no indication for accumulation. According to the abiotic degradation study, transfluthrin is hydrolytically stable at 25 °C, pH 5 and 7. The liver is the main organ responsible for metabolism. The benzylmethylene moiety is predominantly metabolized to tetrafluorobenzoic acid and the glucuronic acid conjugate of tetrafluorobenzyl alcohol. The carboxyl moiety is probably metabolised to dichlorochrysanthemic acid (DCCA). Radioactivity was found in the milk.

10 EVALUATION OF HEALTH HAZARDS

The mammalian toxicity studies of transfluthrin were assessed in the Assessment Report (March 2014), addenda and Proposed Decision of the Netherlands prepared in the context of the approval under Regulation (EU) No 407/2014. Studies considered valid in the CAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. All studies were carried out under GLP unless indicated otherwise. The non-GLP studies were range-finding studies or mechanistic studies. Other than the mechanistic studies, all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries where relevant. In addition to the studies presented in the assessment report of transfluthrin an additional oral acute toxicity study in rat was included which was identified as a 'non-key study' in the assessment report. Inclusion of this study in this CLH dossier is considered to provide a complete overview of the data relevant for classification.

Acute toxicity

10.1 Acute toxicity - oral route

Table 9: Summary table of animal studies on acute oral toxicity

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity in the mouse (OECD 401 (1981))	Mouse, NMRI (SPF-Han), 5 mice/sex/group	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5%	Males: 100, 160, 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw Females: 100, 250, 500, 630, 710, 1000 and 5000 mg/kg bw Single exposure, 14 days post exposure period	583 mg/kg bw	Doc. IIIA/Section A6.1.1
Acute oral toxicity in the rat (OECD 401 (1981))	Rat, SPF-bred Wistar rats, strain Bor: WISW (SPF-Cpb), 5 rats/ sex/ group	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5%	Males and females: 100, 1000, 2500 and 5000 mg/kg bw	> 5000 mg/kg bw	Study A6.1.1-02 Study not included in the CAR since it was considered a 'non-key study'.

Table 10: Summary table of human data on acute oral toxicity

No data available.

Table 11: Summary table of other studies relevant for acute oral toxicity

No data available.

10.1.1 Short summary and overall relevance of the provided information on acute oral toxicity

An acute oral toxicity study is available in which mice were exposed to transfluthrin (Doc. IIIA/Section A6.1.1). This study was performed according to OECD 401 (1981) and is considered GLP compliant.

A single dose of the test material made up in polyethylene glycol E 400 was administered by gavage to groups of fasted male and female NMRI (SPF-Han) mice at doses of 100, 160 (male only), 250, 500, 630, 710, 1000, 1600 (male only) and 5000 mg/kg bw. No mortality occurred at 100 and 160 mg/kg bw. At higher dose levels of 250, 500, 630, 710, 1000, 1600 and 5000 mortality was 1/10, 2/10, 6/10, 5/10, 8/10, 5/5 and 9/10, respectively. Of the animals that died at doses of 250 mg/kg bw or higher, most animals died within 24h after dosing. At 160 mg/kg and higher, the symptoms observed point to an effect of the test compound on the nervous system and consisted of: apathy, tremor, prostration (250 mg/kg bw), spasmodic tremor, dyspnoea, and bristling coats (from 250 mg/kg bw). These symptoms were apparent for a maximum of five days after administration and disappeared rapidly during the observation period. A dose of 100 mg/kg bw appeared to be well tolerated with no symptoms. No effects on body weight gain were observed. Animals that died during the study had some organs that were pale, patchy and/or distended. The LD_{50} was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females.

In addition to the study in mice, also an acute oral toxicity study in rats is available (study A6.1.1-02). This study was performed according to OECD 401 (1981) and is considered GLP compliant.

A single dose of transfluthrin dissolved in polyethylene glycol E 400 was administered by gavage to male and female rats (SPF-bred Wistar rats, strain Bor: WISW [SPF-Cpb]) at dose levels of 100, 1000, 2500 and 5000 mg/kg bw. In males no mortality occurred. In females the mortality was 1/5 at a dose of 5000 mg/kg bw. The respective animal died within one day following transfluthrin exposure and showed slightly patchy and slightly distended lungs, patchy kidneys and a very reddened glandular stomach. In the other animals, sacrificed at the end of the observation, no gross pathological findings were observed. No effects on body weight were observed. Clinical signs suggest an effect on the nervous system and included apathy, tremor, bristling coats and (females only) spasmodic posture in males and females dosed 1000 mg/kg bw and above occurring after 90 to 120 minutes following exposure. Other clinical signs included spasmodic tremors observed a few hours following treatment with 2500 mg/kg bw/day and above in both sexes except for females in the 5000 mg/kg bw/day treatment group, accelerated respiration in males and females 3 days after treatment with 5000 mg/kg bw and spastic gait in females 4 hours after treatment with 5000 mg/kg bw. These effects were all found to be reversible. Based on the results observed, the LD50 was calculated to be >5000 mg/kg bw for male and female rats.

10.1.2 Comparison with the CLP criteria

A clear difference was observed in acute oral toxicity in rats and mice. Both studies are comparable and of good quality. There is no information available showing which species is relevant to humans. Therefore, in line with the CLP guidance (version 5.0) paragraph 3.1.2.3.2 the most sensitive species is used to derive the classification. Based on the available oral acute toxicity study in mice, a LD_{50} of 583 mg/kg bw was derived (male mice). According to Regulation No. (EC) 1272/2008 a substance should be classified as acute toxic category 4 if the LD50 is within the limits $300 < ATE \leq 2000$. Currently, transfluthrin is not classified for oral acute toxicity and a change of the current classification is thus proposed for acute oral toxicity.

10.1.3 Conclusion on classification and labelling for acute oral toxicity

Classification with acute oral toxicity category 4, harmful if swallowed (H302) is proposed for transfluthrin with an ATE of 583 mg/kg bw.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

For the oral LD_{50} two studies in mice and rats were available and reported for transfluthrin. Both studies were performed according to OECD TG 401 (1981) and considered GLP compliant.

***Table**: animal studies on acute oral toxicity (Table 9 of the CLH dossier)*

Method, guideline, deviations if any	Species, strain, sex, no/group	Test substance,	Dose levels, duration of exposure	Value LD_{50}	Reference
Acute oral toxicity in the mouse (OECD TG 401 (1981))	Mouse, NMRI (SPF-Han), 5 mice/ sex / group	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5%	Males: 100, 160, 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw Females: 100, 250, 500, 630, 710, 1000 and 5000 mg/kg bw Single exposure, 14 days post exposure period	Males: 583 mg/kg bw Females: 688 mg/kg bw	Doc. IIIA/Section A6.1.1
Acute oral toxicity in the rat (OECD 401 (1981))	Rat, SPF-bred Wistar rats, strain Bor: WISW (SPF-Cpb), 5 rats/ sex/ group	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5%	Males and females: 100, 1000, 2500 and 5000 mg/kg bw	> 5000 mg/kg bw	Study A6.1.1-02 Study not included in the CAR since it was considered a 'non-key study'.

A single dose of transfluthrin was administered in polyethylene glycol E 400 by gavage to male and female mice at doses of 100, 160 (male only), 250, 500, 630, 710, 1000, 1600 (male only) and 5000 mg/kg bw. No mortalities occurred at 100 and 160 mg/kg bw. At 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw mortality was 1/10, 2/10, 6/10, 5/10, 8/10, 5/5 and 9/10, respectively. Most animals died within 24 h after dosing. At 160 mg/kg bw and above the animals showed signs of toxicity (apathy, tremor, prostration, spasmodic tremor, dyspnoea and bristling coats) until five days after treatment. No effects occurred at 100 mg/kg bw. The LD_{50} was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females.

In male and female Wistar rats a single transfluthrin dose was dissolved in polyethylene glycol E 400 and was administered by gavage at dose levels of 100, 1000, 2500 and 5000 mg/kg bw. One of 5 females of the top dose group died. The animals showed symptoms like apathy, tremor, bristling coats and spasmodic posture until 120 minutes after treatment. The LD_{50} was calculated to be above 5000 mg/kg bw for males and females.

Comments received during consultation

One Member State Competent Authority (MSCA) supported the classification as Acute Tox 4; H302.

Assessment and comparison with the classification criteria

Currently, transfluthrin is not classified for oral acute toxicity.

There was a clear species difference in acute oral toxicity in rats and mice. Both studies were performed similarly. There is no information which species might be more relevant for humans, therefore the more sensitive species (male mice) will be used to derive classification. A substance should be classified as acute toxic category 4 if the LD_{50} is within the limits 300 < ATE ≤ 2000 mg/kg bw.
RAC agrees with the dossier submitter's (DS) conclusion to **classify transfluthrin as acute oral toxicity 4; H302 (harmful if swallowed) with an ATE of 580 mg/kg bw** (rounded).

10.2 Acute toxicity - dermal route

Not evaluated in this dossier.

10.3 Acute toxicity - inhalation route

Not evaluated in this dossier.

10.4 Skin corrosion/irritation

Table 12: Summary table of animal studies on skin corrosion/irritation

Method, guideline, deviations if any	**Species, strain, sex, no/group**	**Test substance,**	**Dose levels duration of exposure**	**Results -Observations and time point of onset -Mean scores/animal -Reversibility**	**Reference**
Skin irritation study in the rabbit (OECD 404 (1981))	Rabbit, HC:NZW, 3 females	NAK 4455 (transfluthrin), 95.0%	Duration of exposure: 4 h Postexposure period: 1 week	No erythema or oedema was observed at any timepoint. Transfluthrin is not a skin irritant.	Doc. IIIA/Section A6.1.4/01

Table 13: Summary table of human data on skin corrosion/irritation

No data available.

Table 14: Summary table of other studies relevant for skin corrosion/irritation

No data available.

10.4.1 Short summary and overall relevance of the provided information on skin corrosion/irritation

Transfluthrin is currently classified as a skin irritant (H315). The information on which the existing classification was based is not available.

Transfluthrin was tested for acute skin irritation in rabbits (Doc. IIIA/Section A6.1.4/01). The test material was applied as a single dose (0.5 mL) to a "hypoallergen" dressing (treated area ~ 6 cm2); the dressing was applied to the clipped flank of three New Zealand White rabbits. The opposite flank was treated the same way, but water was applied to the dressing. Dressing were fastened with semiocclusive elastic adhesive tape and removed after 4 hours. Treated areas were washed with water.

Dermal reactions were observed 1, 24, 48, 72 hours and 1 week after removal of the dressings and scored in accordance with the Draize scale.

No erythema or oedema was observed at any timepoint.

In addition to the acute skin irritation study, a 21-day dermal study in rabbits is available (Doc. IIIA/ Section A6.3.2). Based on this study it was concluded that in the majority of animals effects were found at 1000 mg/kg bw/day but these effects included only minor localised effects at the skin application site. Local effects included redness, scaling, encrustation, swelling, red patches, increased skin fold thickness, thickening of the epidermis, and hyperkeratosis. Only skin redness was scored in accordance with the Draize scores at 24, 48 and 72 hours resulting in scores of 0.3, 0.7, 1.3 and 0.3, 0.8, 0.4 for males and females, respectively, which was completely reversible. Signs of inflammation were also completely reversible within the observation period.

10.4.2 Comparison with the CLP criteria

According to Regulation No. (EC) 1272/2008 a substance should be classified as skin irritant if:

(1) Mean score of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or

(2) Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling; or

(3) In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.

Transfluthrin does not fulfil the criteria for skin irritation as no erythema and no oedema were observed at any time point in the acute dermal irritation study. This conclusion is strengthened by the outcome of a 21-day dermal study in rabbits (see section 10.4.1 and 10.12).

10.4.3 Conclusion on classification and labelling for skin corrosion/irritation

Based on the available study it is proposed to remove the current classification as Skin Irrit. 2. Data is conclusive but not sufficient for classification.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

Transfluthrin was tested for acute skin irritation in an OECD TG 404 (1981) study in rabbits under semi-occlusive conditions. The test material was applied as a single dose (0.5 mL) to the clipped flank of three New Zealand White rabbits for 4 hours. The opposite flank was treated the same way but with water applied to the dressing. Treated areas were washed with water. Reactions were observed 1, 24, 48, 72 hours and 1 week after removal of the dressings and scored in accordance with the Draize scale. No erythema or oedema was observed at any time point.

In a OECD TG 410 21-day dermal study in rabbits (Doc. IIIA/ Section A6.3.2) minor localised effects at the skin application site were found in most animals at 1000 mg/kg bw. Local effects included redness, scaling, encrustation, swelling, red patches, increased skin fold thickness, thickening of the epidermis, and hyperkeratosis. Skin redness was scored at 24, 48 and 72 hours resulting in scores of 0.3, 0.7, 1.3 and 0.3, 0.8, 0.4 for males and females, respectively. These effects were fully reversible.

Comments received during consultation

One MSCA supported the removal of Skin Irrit. 2; H315.

Assessment and comparison with the classification criteria

Transfluthrin is currently classified as a skin irritant (H315). However, the reason for the classification is unknown.

The available studies for transfluthrin do not fulfil the criteria for skin irritation as no erythema and no oedema were observed at any time point in the acute dermal irritation study. This conclusion is strengthened by the outcome of a 21-day dermal study in rabbits.

RAC supports the dossier submitter's proposal to **remove the current classification as Skin Irrit. 2; H315.**

10.5 Serious eye damage/eye irritation

Not evaluated in this dossier.

10.6 Respiratory sensitisation

Not evaluated in this dossier.

10.7 Skin sensitisation

Not evaluated in this dossier.

10.8 Germ cell mutagenicity

Not evaluated in this dossier.

10.9 Carcinogenicity

Table 15: Summary table of animal studies on carcinogenicity

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
2-year oral rat study OECD 453 (1981) US EPA FIFRA § 83-5 (1984)	Transfluthrin, mixed batch no: 130187, purity 95.0% Rat, Wistar; Bor:WISW (SPF-Cpb), 70 rats/sex/group Duration of treatment: 25 months Dose: Food 0, 20, 200, 2000 ppm, equivalent to: Males: 0, 1.0, 9.9, 100.4 mg/kg bw-day Females: 0, 1.4, 13.6, 142.1 mg/kg bw-day	No treatment induced changes in behaviour, appearance, mortality, food or compound intake was observed. No treatment related damage to the eye was observed. The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occur in both sexes exposed to 2000 ppm (100.4/142.1 mg/kg bw/d) and likely begins at 200 ppm (9.9/13.6 mg/kg bw/d). The urinary bladder urothelial hyperplasia, thyroid follicular hyperplasia and increased cuboidal cells (m+f) and urinary bladder tumours (papilloma and carcinoma) were observed at 2000 ppm (equal to 100.4 mg/kg bw/day).	Doc IIIA/Section A 6.7/01
2-year oral mouse study OECD 451 (1981) US EPA FIFRA §	Transfluthrin, Mixed batch no: 250987, purity 94.5-95% Mice, B6C3F1, 60 mice/sex/group (+ extra 10 mice/sex/group for 0 and 1000 ppm groups) Duration of treatment: 24	Mortality was unaffected by treatment. No treatment induced changes in behaviour, or appearance were observed. No treatment related effects were seen on food or water consumption. The results from the haematological and clinical chemistry studies combined with histopathology suggest that liver damage occur in both sexes exposed to 1000 ppm (199.5/279.0 mg/kg bw/d) and may begin at 100 ppm (33.3 mg/kg bw/d) in females. There was an increase in hepatocellular adenoma in	Doc IIIA/Section A 6.7/02

Method, guideline, deviations if any, species, strain, sex, no/group	**Test substance, dose levels duration of exposure**	**Results**	**Reference**
82-2 (1984)	months Dose: Food 0, 10, 100, 1000 ppm, equivalent to: Males: 0, 2.1, 19.7, 199.5 mg/kg bw-day Females: 0, 3.1, 33.3, 279.0 mg/kg bw-day	females at 1000 ppm (equal to 279 mg/kg bw/day) (13/50). In the females at 1000 ppm (equal to 279 mg/kg bw/day) there was a treatment-related increase in haemangiosarcomas in the spleen (2/50), adenomas in the Harderian gland (8/50) and sarcomas of the subcutis (2/50).	

Table 16: Summary table of human data on carcinogenicity

No data available.

Table 17: Summary table of other studies relevant for carcinogenicity

Type of study/data	**Test substance,**	**Relevant information about the study (as applicable)**	**Observations**	**Reference**
Mechanistic study - determination of transitional cell proliferation in the urinary bladder (no guideline)	Transfluthrin, Batch no. 8169 79301, purity 95.8%	Organism/ species: Rat, Wistar HsdCpb:wu, females Number of animals per group: Negative control, 1 week – 20 Negative control, 4 weeks – 30 Transfluthrin, 1 week – 20 Transfluthrin, 4 weeks – 30 Positive control, 1 week – 10 Positive control, 4 weeks – 10 Duration of treatment: 1 or 4 weeks Dose: 5000 ppm, food, 327 mg/kg bw/day Controls: Negative control - Altromin 1321 diet with 1% peanut oil Positive control – 100 ppm sodium cacodylic acid (NaC) in Altromin diet, 7.38 mg/kg bw/day	Treatment-related effects on the urinary system: increased urine excretion in some animals, decreased urine calcium concentration and excretion, increased protein excretion, and increased kidney weights at 4 weeks. The treatment had no effect on survival, body weight, and food consumption. The mean BrdU labelling (marker for cell proliferation) increased 3.7 fold over controls at 5 weeks in the treated animals. The positive control induced a 2.4-fold increase. There was no correlation between BrdU labelling and TFBA metabolite concentration in the urine.	Section IIIA6.10/01
Mechanistic study - Clarification for bladder tumours	Tetrafluobenzoic acid (TFBA, NAK 4723), a metabolite of	Organism/ cell type: Rat bladder epithelial explant cultures and a permanent fibroblast cell line of the mouse (3T3) (cytotoxicity)	Cytotoxicity was limited in the 3T3 cell line, with an IC50 of >1000µg/ml In the primary explant	Doc. IIIA/ Section A6.10/02

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
(no guideline)	Transfluthrin (NAK 4455)	Positive control: Mitomycine (purchased from Serva, Cat. No. 29805) Concentrations: 0, 10, 30, 100, 300 and 1000µg/ml	cultures of rat bladder epithelial cells, TFBA was not cytotoxic up to 100µg/ml. The growth was inhibited at 300µg/ml, and explants were dead at 1000µg/ml. At 1000µg/ml TFBA, DNA content was reduced to background levels.	
Mechanistic Study – 4/13 week oral toxicity in rats and mice (no guideline)	Transfluthrin, lot/ batch number ABIDTBN019, purity 99.6% w/w	Organism/ species: Rat and Mouse, Wistar Hanover IGS [CRL: WI (Han)] rats (nulliparous and nonpregnant) B6C3F1 mice rats (nulliparous and nonpregnant) Number of animals per group: 10 animals/group Duration of treatment: 4 weeks for rat groups 1, 3, 5, 6, 7 and both mouse groups, 13 weeks for rat groups 2, 4. Dose: Rats: 4 weeks: 0, 180, 454, 0 and 542 mg/kg bw 13 weeks: 0, 435 mg/kg bw, Mice: 4 weeks: 0, 401 mg/kg bw Controls: vehicle, plain Altromin 1321diet or Altromin 1321 diet + 1.25% NH_4Cl	Effects on the bladder epithelium were observed by SEM in the rat 4 week 454 mg/kg/bw Altromin 1321 diet + 1.25% NH4Cl group, and the 180 mg/kg/bw 13 week group, but not in the 180 mg/kg bw 4 week group. No increase in hyperplasia or BrdU labelling index was observed in any group. The SEM examination of the bladder surface of the mice could not be interpreted due to extensive changes which might be related to the high pH caused by the Altromin diet	Section A 6.10/03
Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human (no guideline)	Transfluthrin: Batch No. ABIDTBN019, purity 99.6%	Organism/ cell type: Liverbeads ™ from rat, mouse, dog and human Controls: Positive (SDS) and negative (DMSO) controls were also assayed in this experiment. Concentration: 2 concentrations of Transfluthrin were tested: 25 and 250 µM (two wells per concentration were used).	Transfluthrin was well metabolised (no detectable parent compound in any species after 24 hours). The major metabolite was TFB alcohol in all species, being further metabolized into the glucurono-conjugated TFB alcohol. A minor metabolite was TFBA, which was detected in the rat and mouse only.	Section A 6.10/04
The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines (no guideline)	Transfluthrin: Batch No. ABIDTBN019, purity 99.6% Tetrafluorobenzoic acid: Batch No. 950627ELB01, purity 99.0%	Organism/ cell type: MYP3 rat urothelial cell, 1T1 human urothelial cell line	Transfluthrin: Due to limitations of solubility, it was not possible to prepare a stock solution at a high enough concentration to determine the LC50 of transfluthrin for rat or human urothelial cells. TFBA: The LC50 of TFBA	Section A 6.10/05

Type of study/data	Test substance,	Relevant information about the study (as applicable)	Observations	Reference
			for the rat urothelial cell line MYP3 was determined to be 2.25 mM (r2=0.8564). The LC50 of TFBA for the human urothelial cell line 1T1 was determined to be 2.43 mM (r2=0.8715).	
Preliminary concentration range finding study in cultured female B6C3F1 mouse hepatocytes	Transfluthrin: Batch No. PMLO000319 Purity: 98.9%	Organism/ cell type: one B6C3F1 mouse, Primary monolayer cultures of hepatocytes Concentrations: 0.03, 0.1, 0.3, 1, 3, 10, 30, 100, 300, 500, 600, 900 and 1000 µM transfluthrin	The results demonstrate no statistical significant levels of cytotoxicity. At 300 µM and above, transfluthrin was found not to completely dissolve in the culture medium which could explain the lack of cytotoxicity at the highest concentrations tested.	Annex 1, Section 3.9.4.6 Study 6
Enzyme, mRNA and DNA synthesis induction in cultured females B6C3F1 mouse hepatocytes	Transfluthrin: Batch No. PMLO000319 Purity: 98.9%	Organism/ cell type: Five B6C3F1 mice, Primary monolayer cultures of hepatocytes Controls: phenobarbital, EGF, and DMSO Concentrations: 30, 100, 300 and 1000 µM transfluthrin	Transfluthrin increases the expression of some P450 mRNA levels, but strongly inhibits cytochrome P450 enzyme activity. Transfluthrin induced a statistically significant increase in replicative DNA synthesis, however, no increase was observed for phenobarbital.	Annex 1, Section 3.9.4.7 Study 7
Preliminary concentration range finding study in cultured human hepatocytes from three different cultures	Transfluthrin: Batch No. PMLO000319 Purity: 98.9%	Organism/ cell type: Three human donors, Primary monolayer cultures of hepatocytes Concentrations: 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300 µM transfluthrin	Statistical significant levels of cytotoxicity occurred in cells from 2 out of 3 donors at transfluthrin levels of 300 µM	Annex 1, Section 3.9.4.8 Study 8
Enzyme, mRNA and DNA synthesis induction in cultured females human hepatocytes	Transfluthrin: Batch No. PMLO000319 Purity: 98.9%	Organism/ cell type: Three human donors, Primary monolayer cultures of hepatocytes Controls: WY 14643, EGF Concentrations: 3, 10, 30 and 100 µM transfluthrin	Transfluthrin was cytotoxic at 100 µM. BQ enzyme activity (CYP3A) was significantly decreased. No clear effect was observed on gene activity. Transfluthrin did not induce an increase in DNA synthesis.	Annex 1, Section 3.9.4.9 Study 9

10.9.1 Short summary and overall relevance of the provided information on carcinogenicity

Carcinogenicity study in mice

In an oncogenicity study in mice, 60 animals/sex/dose were exposed for 24 months via food to doses of 0, 10, 100, 1000 ppm transfluthrin (equivalent to: Males: 0, 2.1, 19.7, 199.5 mg/kg bw/day, Females: 0, 3.1, 33.3, 279.0 mg/kg bw-day), of which 10 animals/sex/dose were allocated for interim sacrifice at 12 months. Body weights of females in the high dose group were statistically significantly increased (≤ 10%) over controls except during the last part of the study. The liver was the target organ, as shown by changes in haematology and clinical chemistry, as well as histopathology. Slight changes were observed at the mid dose, which became pronounced at the high dose and included liver weight increase and hypertrophy of the periacinal hepatocytes.

High dose group females had statistically significant increased levels of hepatocellular adenomas (13/50 versus 4/50 in the controls). No increase in hepatocellular adenomas was observed in males and there was no increase in the number of carcinoma in either sex compared to the control group. It should be noted that the B6C3F1 strain of mouse is known to have a high incidence of spontaneously-occuring liver tumours.

Also other pyrethroids have been associated with the production of hepatocellular tumours in rats and/or mice. In some cases, the mode of action was shown to be related to the induction of P450 isozymes through CAR activation. This mechanism is often considered not relevant to humans. Additional mechanistic studies have been performed with transflutrin to assess whether this also applies to transfluthrin. These mechanistic studies are discussed in the section below.

Other neoplastic lesions observed in the carcinogenicity study in mice were increased incidences in haemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50), and sarcomas of the subcutis (2/50) in females at 1000 ppm (equal to 279 mg/kg bw/day) (Doc IIIA/Section A 6.7/02).

Considering the haemangiosarcomas in the spleen, the incidence was found to be above the historical control value. The historical control data was obtained from 13 two-year studies conducted at the same laboratory with the B6C3F1 mouse in the years following the transfluthrin study. However, the data also show that this finding is only slightly outside the performing laboratory historical control data (4% vs. 2%). The effect observed was confined to one sex and was not statistically significant. A haemangiosarcoma is vascular in origin, therefore if this tumour is truly treatment-related, then an increased incidence of this tumour type would be expected in other organs. There is no increase in the incidence of haemangiosarcomas in any other organ, nor is there an increase in the cumulated incidence (4-0-2-3) of this tumor in all organs. Therefore, the very slight increase of haemangiosarcomas in the spleen in the transfluthrin study, compared to concurrent controls, is likely to be incidental and unrelated to treatment.

The Harderian gland is not present in humans and has therefore no relevance for human risk. Moreover, it can be clearly stated that combining hyperplastic and neoplastic (benign and malignant) lesions does not show an increase of the Harderian gland lesions (6-6-6-8) in the present study, that all adenomas occurred only unilaterally i.e. in only one sex as single events, there was no increased incidence of this lesion in males, there was no progression from benign to malignant and the most serious lesion ‘adenocarcinoma’ was reported in the control group.

Two tumours occurred in the skin/subcutis of two high-dose females, which were classified as “Sarcoma NOS” (not otherwise specified) by the Study Pathologist. Both were found as gross lesions in the flank region. In animal N° 450, which had to be euthanized on day 462, the single white/beige lesion had a diameter of 3 cm and was of elastic structure. In contrast, in animal N° 471, which died at the terminal phase of the study on day 715, several nodes were observed, which had varying colors, structure and size up to 3.8 x 2.0 x 1.3 cm. Both lesions were re-examined. Since the lesion of the animal N° 450 was composed of irregular spindeloid tissue fibers of uncertain origin and intermingled with various amounts of myxoid ground substance, the classification as “Sarcoma NOS” seems appropriate. The lesion of animal N° 471 was differently composed and consisted in the main area of neoplastic cells with large nuclei and evidence of striation of the cytoplasm, it could have therefore been more appropriately classified as most likely to be a “rhabdomyosarcoma” even though special staining methods, such as PTAH, were not applied. Since both lesions occurred in non-protocol locations, historical control data are naturally not available. Rhabdomyosarcomas represent a rare lesion, and there is only one case in the RITA Database among 922 female B6C3F1 mice of a study which was performed between August 1995 and September 1997.

Results from carcinogenicity study in mice

	Control data								**dose-response + /**	
	study		**low dose**		**medium dose**		**high dose**			
Parameter	m	f	m	f	m	f	m	f	m	f
Number of animals examined	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**		
Mortality	**9**	**5**	**2**	**2**	**8**	**11**	**8**	**6**	-	-
Clinical signs	-	-	-	-	-	-	-	-	-	-
Body weight	-	-	-	-	-	-	-	↑**	-	-
Food consumption	-	-	-	-	-	-	-	-	-	-
Overall tumour incidence (%):	**50**	**58**	**42**	**54**	**50**	**56**	**40**	**74**		
No. of animals with neoplasms	**25/50**	**29/50**	**21/50**	**27/50**	**25/50**	**28/50**	**20/50**	**37/50**	-	-
No. of animals with benign neoplasms	**14/50**	**13/50**	**10/50**	**14/50**	**11/50**	**9/50**	**9/50**	**19/50**	-	-
No. of animals with malignant neoplasms	**10/50**	**10/50**	**9/50**	**11/50**	**11/50**	**16/50**	**7/50**	**10/50**	-	-
No. of animals with > 1 neoplasm	**3/50**	**9/50**	**3/50**	**5/50**	**6/50**	**6/50**	**7/50**	**17/50**	-	-
Liver										
Eosinophilic focus of cellular alteration (total)	**0/50**	**0/50**	**0/50**	**0/50**	**0/50**	**1/50**	**1/50**	**4/50**	-	-
Hepatocellular adenoma	**5/49**	**4/50**	**4/50**	**2/48**	**5/50**	**2/50**	**5/50**	**13/50***	-	-
Carcinoma	**5/49**	**2/50**	**8/50**	**2/48**	**7/50**	**4/50**	**7/50**	**4/50**	-	-
Non-neoplastic changes										
Nodule	**10/50**	**7/50**	**13/50**	**4/50**	**13/50**	**5/50**	**12/50**	**15/50**	-	-
Hypertrophy of periacinal hepatocytes (interim)	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**0/10**	**10/10**	**6/10**	-	-
Hypertrophy of periacinal hepatocytes (final)	**0/50**	**0/50**	**0/50**	**0/50**	**0/50**	**0/50**	**38/50*** **	**26/50*** **	-	-
Absolute weight (interim 12 month)	-	-	-	-	-	-	↑**	↑**	-	-
Absolute weight (final 24 month)	-	-	-	-	↑*	↑	↑**	↑**	+	+
Relative weight (interim 12 month)	-	-	-	-	-	↓**	↑**	-	-	-
Relative weight (final 24 month)	-	-	-	-	-	-	↑**	↑**	-	-

*p <0.05, ** p<0.01, *** P<0.001, - Not significantly different than control.

Carcinogenicity study in rat

In the rat carcinogenicity study, 60 animals/sex/dose were exposed for 25 months to doses of 0, 20, 200, 2000 ppm transfluthrin in food (Males: 0, 1.0, 9.9, 100.4 mg/kg bw-day, Females: 0, 1.4, 13.6, 142.1 mg/kg bw-day). Additional 10 animals/sex/dose were allocated for interim sacrifice at 12 months.

The target organs were the liver and kidney in both sexes. Slight effects were observed at the mid dose and these became more pronounced at the high dose. The non-neoplastic effects are discussed in more detail in the STOT RE section.

At the high dose, there was an increased incidence of urinary bladder urothelial hyperplasia, as well as an increased incidence of urothelial tumours papilloma and carcinoma in both sexes. The tumour incidences were low (3 papilloma and 3 carcinoma in both sexes together), and no bladder tumours were observed in any of the other dose groups. Thyroid follicular hyperplasia and increased cuboidal cells in the thyroid were seen at 2000 ppm (equal to 100.4 mg/kg bw/day) (Doc IIIA/Section A 6.7/01). In male rats, also an increased incidence of hepatocellular adenomas was found at all dose levels tested, but increased incidences were not significantly different from the control and there was no dose related response. For details please refer to the table below.

The hypothesized mode of action for transfluthrin-induced rat bladder tumors is related to cytotoxicity and regenerative proliferation induced by the primary metabolite tetrafluorobenzoic acid (TFBA), ultimately leading to the production of tumors. A detailed description of the mode of action based on mechanistic data is presented below.

There was a slight increase in incidences of thyroid follicular hyperplasia in the mid and high dose and of cuboidal cells in the thyroid, but the observed effects were not significantly different from the control (see table below). Moreover, an increased incidence of tumors in the thyroid was absent. The effect on cuboidal cells was most prevalent in the male high-dose group and according to the study authors the effect was not regarded as hypertrophy since it was considered to be within the range of physiological deviation. Hypertrophy of the follicle epithelium was found to be partly reversible. These effects on the thyroid are probably secondary to liver hypertrophy due to elevated foreign substance metabolism.

Results from carcinogenicity study in rat

	Control data study		**low dose**		**medium dose**		**high dose**		**dose-response +/**	
Parameter	m	f	m	f	m	f	m	f	m	f
Number of animals examined	**59**	**59**	**60**	**60**	**59**	**60**	**58**	**60**		
Mortality	**2**	**1**	**3**	**8**	**8**	**6**	**4**	**5**	**-**	**-**
Clinical signs	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**		
Body weight	**-**	**-**	**-**	**-**	**↑***	**-**	**↑***	**↓***		
Food consumption	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**		
Overall tumour incidence (%):	**44**	**64**	**55**	**72**	**56**	**53**	**62**	**50**		
No. of animals with neoplasms	**26/59**	**38/59**	**33/60**	**43/60**	**33/59**	**32/60**	**36/58**	**30/60**	**+**	**-**
No. of animals with benign neoplasms	**23/59**	**38/59**	**22/60**	**34/60**	**25/59**	**33/60**	**30/58**	**30/60**	**+**	**-**
No. of animals with malignant neoplasms	**2/59**	**3/59**	**4/60**	**4/60**	**3/59**	**3/60**	**2/58**	**5/60**	**-**	**-**
No. of animals with > 1 neoplasm	**1/59**	**3/59**	**3/60**	**7/60**	**0/59**	**2/60**	**0/58**	**2/60**	**-**	**-**
Liver										
Hepatocellular adenoma	**0/59**	**0/59**	**3/60**	**0/60**	**2/59**	**0/60**	**3/58**	**0/60**	**-**	**-**
Carcinoma	**1/59**	**0/59**	**0/60**	**0/60**	**0/59**	**0/60**	**0/58**	**0/60**	**-**	**-**
Non-neoplastic changes										
Swollen/thickened/ enlarged	**0/59**	**0/59**	**4/60**	**0/60**	**0/59**	**2/59**	**5/58**	**3/60**	**-**	**-**
Nodule	**0/59**	**0/59**	**3/60**	**0/60**	**2/59**	**0/60**	**3/58**	**0/60**	**-**	**-**
Absolute weight (interim 12 month)	**-**	**-**	**-**	**-**	**-**	**-**	**↑****	**↑****		
Absolute weight (final 24 month)	**-**	**-**	**-**	**-**	**-**	**-**	**↑**	**↑***		
Kidney										

Tumour (lipomatous)	**0/59**	**0/59**	**0/60**	**1/60**	**0/59**	**0/60**	**2/58**	**0/60**		
Carcinoma	**0/59**	**0/59**	**1/60**	**0/60**	**0/59**	**0/60**	**0/58**	**0/60**		
Non-neoplastic changes										
Glomerulonephrosis	**45/59**	**11/59**	**47/60**	**18/60**	**53/59**	**21/60**	**56/58**	**13/60**		
Pigment deposition	**41/59**	**33/59**	**41/60**	**40/60**	**53/59**	**54/60**	**58/58**	**59/60**		
Absolute weight (interim 12 month)	-	-	-	-	↑	↑*	↑*	↑*		
Absolute weight (final 24 month)	-	-	-	-	↑**	↑*	↑**	↑		
Urinary bladder										
Papilloma	**0/58**	**0/59**	**0/59**	**0/60**	**0/58**	**0/60**	**2/57**	**1/60**		
Carcinoma	**0/58**	**0/59**	**0/59**	**0/60**	**0/58**	**0/60**	**1/57**	**2/60**		
Non-neoplastic changes										
Hyperplasia	**2/59**	**0/59**	**1/60**	**1/60**	**2/59**	**2/60**	**7/58**	**10/60**	+	+
Thyroid										
C-cell adenoma	**2/58**	**3/59**	**2/60**	**5/60**	**1/59**	**2/60**	**2/58**	**2/59**	-	-
Follicular adenoma	**3/58**	**1/59**	**1/60**	**0/60**	**1/59**	**1/60**	**2/58**	**1/59**	-	-
Follicular adenocarcinoma	**0/58**	**0/59**	**0/60**	**1/60**	**0/59**	**0/60**	**1/58**	**0/59**	-	-
Non-neoplastic changes										
Follicular hyperplasia	**0/59**	**0/59**	**0/60**	**0/60**	**3/59**	**1/60**	**4/58**	**2/60**	-	-
Increased cuboidal cells	**1/10**	**1/10**	**2/10**	**2/10**	**2/10**	**1/10**	**7/10**	**2/10**	-	-

*p <0.05, ** p < 0.01,- Not significantly different than control.

Mechanistic studies: bladder tumours

Several studies are available that investigate the mechanism behind the induction of bladder tumours by transfluthrin (see table 17 for details):

- A four week in vivo study (Section IIIA6.10/01) in which female rats were exposed to 5000 ppm (327 mg/kg bw/d) of transfluthrin for 1 and 4 weeks. The proliferation of urothelial cells was determined with BrdU (5'-bromo-2'-deoxyuridine) labelling. The main findings at four weeks were a statistically significant increase in absolute kidney weight and an 3.7-fold increase in BrdU labelling index. However, there was no correlation between BrdU labelling and the concentration TFBA metabolite in the urine (concentrations TFBA not given).
- A cytotoxicity study of TFBA in the 3T3 cell line and rat bladder epithelial explant cultures (Doc. IIIA/ Section A6.10/02). TFBA was not cytotoxic in the 3T3 cell line with and IC50 of >1000µg/ml. The growth of primary explant cultures of rat bladder epithelial cells was inhibited at 300µg/ml, and explants were dead at 1000µg/ml.
- An in vivo study in rats and mice to the effect of transfluthrin on the bladder epithelium. Effects on the bladder epithelium were observed by SEM in the rat 4 week 454 mg/kg/bw Altromin 1321 diet + 1.25% NH_4Cl group, and the 180 mg/kg/bw 13 week group, but not in the 180 mg/kg bw 4 week group. At 3 weeks, the concentration TFBA in the urine was 571 µg/ml in rats at 180 mg/kg/day, 1065 µg/ml in the 454 mg/kg/bw + 1.25% NH_4Cl group, and 276 µg/ml in mice at 401 mg/kg/day. No increase in hyperplasia or BrdU labelling index was observed in any group. The mouse results could not be interpreted due to extensive changes also in the control group.
- A comparative metabolism study in Liverbeads with cells from rat, mouse, dog and human (Section A 6.10/04). The main metabolites in all species were TFB alcohol and Glucuronide-TFB alcohol. Small fractions of TFBA were found with rat and mouse cells only (0.77-4.46%).
- A comparative cytotoxicity study with TFBA in rat and human urothelial cells (Section A 6.10/05). The LC50 of TFBA was comparable in rat and human cell lines (2.25 vs 2.43 mM).

The registrant argues that the results of these studies suggest urothelial cytotoxicity and associated regenerative proliferation caused by high, sustained urinary concentrations of TFBA as the mechanism of urinary bladder tumour formation in rats exposed for two years to a high dose level of transfluthrin.

This is coupled to the findings in the (sub-)chronic and carcinogenicity studies, in particular the carcinogenicity study in mice and 13-week and 1-year studies in dogs, in which respectively no bladder tumours and no histopathological changes of the bladder were found. Based on these two arguments, the registrant considers the bladder tumours in rats not relevant to humans.

The first mechanistic study in female rats does indeed suggest that transfluthrin induces proliferation of the urothelial cells in rats. However, there was no correlation with the concentration TFBA. TFBA showed cytotoxicity in cell lines at high concentrations, but a rat cell line was no more sensitive than a human cell line (LC50 rat 2.25 mM and LC50 human 2.43 mM). The Liverbead study suggest that the species differences in metabolism are minor, but it is suggested that the amount of TFBA formed in the Liverbeads was much lower than measured in the urine. Unfortunately, the study to the effect on the bladder epithelium in rats and mice had absent urinary MgNH4PO4 crystals in rats, and unusable mouse bladders.

More importantly, the actual TFBA levels are not given for the carcinogenicity study and unknown for humans, which means that the comparison remains hypothetical. Although TBFA was concluded to be a major rat metabolite, it is also not proven that TFBA is the (only) active metabolite, as the studies did not look at the effects of the other metabolites of transfluthrin. Hence, the DS considers the evidence on the human relevance of the bladder tumours in rats inconclusive.

Mechanistic studies: liver tumours

In the mouse carcinogenicity study, transfluthrin promoted liver tumour development in female mice. Also a number of other pyrethroids are associated with production of hepatocellular tumours in rats and /or mice when administered at high doses. Based on the available mutagenicity studies for transflutrin it is unlikely that transfluthrin has a genotoxic mode of action. In addition, data available on structurally related pyrethroids (e.g. epsilon-metofluthrin) suggest a non-genotoxic mode of action for liver tumor formation. For some pyrethroids, the mode of action and the induction of liver changes have been demonstrated to be due to induction of P450 isozymes through constitutive androstane receptor (CAR) activation, similar to what occurs in response to phenobarbital. The mode of action includes the following events: CAR activation, enzyme (CYP 2B) induction, cell proliferation and tumor induction (Yamada et al., 2009).

Two mechanistic studies were performed to investigated whether transfluthrin is indeed a CAR activator:

- A first study investigated the potential of transfluthrin to stimulate cell proliferation and activate several nuclear hormone receptors (aryl hydrocarbon receptor (AhR); constitutive androstane receptor (CAR); pregnane-X-receptor (PXR) and peroxisome proliferator activated receptor (PPARα)) in cultured female B6C3FI mouse hepatocytes (Annex I to the CLH report section 3.9.4.7, study 7). Transfluthrin was not cytotoxic up to the maxium soluble concentration, as indicated by slight precipitation at concentrations of 300 µM and above. Treatment of the mouse hepatocytes with 30-1000 µM transfluthrin resulted in a weak induction of mRNA levels of different cytochrome P450 enzymes, at the dose of 300 µM. However, the expression of Cyp2b10, which is strongly induced by phenobarbital as well as e.g. sulfloxaflor was only mildly but not statistically significant induced by transfluthrin. And vice versa, Cyp 4a10 and Cyp 4a14 were slightly induced by 300 µM transfluthrin, but not by phenobarbital. All doses inhibited the activity of several liver enzymes (EROD, PROD, BROD, BQ) by a factor 5 to 100, in contrast to a clear induction by the positive control phenobarbital. No further analysis was conducted to explain this finding. Transfluthrin induced an increase in replicative DNA synthesis from 100 µM and above. However, the positive control phenobarbital had no effect on replicative DNA synthesis. Based on this study, no clear conclusion can be drawn on the question whether transfluthrin induces CAR activation.

- A second study explored the same parameters in cultured human hepatocytes taken from three different female donors (Annex I to the CLH report section 3.9.4.9, study 9). Treatment of the human hepatocytes resulted again in a dose-dependent inhibition of the liver enzyme BQ (others not tested), which was induced by the positive control WY14643 (PPARα activator). The effects on the expression of mRNA of human CYP enzymes was generally small and showed high variation both between donors and samples from the same donor. Most notable was CYP3A4, which was induced in one donor but not in the others. There was no statistically significant effect on replicative DNA synthesis.

When comparing the response of the mouse and human hepatocytes to transfluthrin with the positive controls phenobarbital for CAR activation and WY14643 for PPARα activation, there are some similarities in the induction of mRNA levels of different cytochrome P450 enzymes, but also various differences.
The most striking difference is the strong inhibition in liver enzyme activity in mice, which is also seen in lesser extent in human cells, while these enzymes are induced by the positive controls. The implications of this finding are unclear. The gene usually associated with CAR activation, Cyp2b10 in mice, was not activated by transfluthrin.
An effect that is supportive of CAR activation is that transfluthrin increases replicative DNA synthesis in mouse hepatocytes, but not in human hepatocytes. However, CAR-activator phenobarbital did not induce an increase, a finding that is contrary to what would be expected. It should also be noted that this induction was observed from a concentration of 100 µM onward. There are no data to show whether this matched the exposure of hepatocytes in vivo.

Unfortunately, there is no study available with humanized CAR/PXR knock-out mice to demonstrate the specificity for the CAR mode of action. As only female mouse hepatocytes were tested, it is unclear whether there are any mechanistic differences between sexes and between mice and rats. This would have been relevant, as it might explain why male mice and rats seem to be less sensitive to the induction of liver tumours by transfluthrin, while they are also sensitive to CAR activators.
Based on these mechanistic studies, no definitive conclusion can be drawn on the mode of action of transfluthrin regarding the liver tumours in female mice.

10.9.2 Comparison with the CLP criteria

According to Regulation EC No 1272/2008 (CLP), Table 3.6.1, classification for carcinogens is based on:

CATEGORY 1: Known or presumed human carcinogens 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:

- o *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.

CATEGORY 2: Suspected human carcinogens The placing of a substance in Category 2 is done on the basis of evidence 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 (see section 3.6.2.2). Such evidence may be derived either from limited(1) evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies.

Classification as Carc. 1A is not justified as no human data are available for transfluthrin.

There are two in vivo carcinogenicity studies available, one in rats and one in mice. The rat study found a slightly increased incidence of urinary bladder tumours in both sexes at the top dose (about 100/150 mg/kg bw/d in males/females). The mechanistic evidence on the human relevance of the bladder tumours in rats is inconclusive. Based on the data available, there is no evidence for a genotoxic mode of action for the effects, as the in vivo micronucleus test was negative. Also thyroid toxicity was observed, specifically thyroid follicular hyperplasia and increased cuboidal cells in the thyroid, but this did not lead to thyroid tumours.

In mice increased incidences in haemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50), hepatocellular adenomas (13/50) and sarcomas of the subcutis (2/50) were observed in high dose females. As the incidences in haemangiosarcomas in the spleen and sarcomas of the subcutis were small and the tumours emerged from different tissues, these findings are likely to be incidental and unrelated to treatment. Also the relevance of the adenomas of the Harderian gland is questionable, as the Harderian gland is not present in humans, all adenomas occurred only unilaterally i.e. in only one sex as single events, there was no increased incidence of this lesion in males, and there was no progression from benign to malignant.

A significant increase in hepatocellular adenomas was found at high dose levels in females.

In addition, at 12 and 24-months, increased incidences of hepatocyte centrilobular hypertrophy was observed in both sexes at the highest dose level, which were associated with increased liver weights at 3, 12 and 24 months. To investigate whether the liver toxicity and hepatocellular adenomas were induced through CAR activation, additional mechanistic studies were conducted with cultured hepatocytes from mouse and human livers.

In these studies, transfluthrin clearly inhibited the activity of various liver enzymes in both mouse and human hepatocytes, which is contrary to what would be expected from a CAR activator. The mRNA levels of some P450 enzymes were increased, but the pattern was only partially in agreement with that of CAR activators.

Transfluthrin increases replicative DNA synthesis in mouse hepatocytes, but not (statistically significant) in human hepatocytes. It is unknown whether there is dose concordance between the concentrations at which this induction was observed in vitro (≤100 μM) and the in vivo concentrations in the liver. Moreover, this finding on its own is not sufficient evidence to conclude transfluthrin is a CAR/PPARα activator, or that the hepatocellular adenomas are not relevant to humans.

Based on these mechanistic studies, no definitive conclusion can be drawn on the mode of action of transfluthrin regarding the liver tumours in female mice.

Based on the available data (in vitro and in vivo) discussed above, increased incidences of several tumors were observed in rats and mice. The occurrence of tumours in two species is generally considered sufficient evidence to classify in Category 1B. However, there are several factors that diminish the strength of evidence.

In rats, only the incidence of bladder tumours was increased, but this increase was not statistically significant. Liver toxicity was observed, but there was no increase in liver tumours, in contrast to the mouse. Dosing might play a role here, as the top dose levels in mg/kg bw/d were about a factor two lower than in the mouse study, but as no higher dose was tested in rats, nor were they included in the mechanistic studies, this remains unknown.

In mice, only the increase in hepatocellular adenoma at high dose females was statistically significant, which is a benign neoplasm. The haemongiosarcomas in the spleen and sarcomas of the subcutis occurred only in two high dose animals each, hence they could very well be incidental. On the other hand, these are relatively rare tumours, and incidences fell respectively marginally outside the historical control or no historical control was available.

Considering the uncertainties, the lack of statistical significance for most tumour types, and the type of tumours that was increased significantly, classification in Category 2 is considered more appropriate than Category 1B.

No classification is not considered justified, as the mechanistic evidence to investigate the human relevance of both the bladder tumours in rats and the hepatocellular adenoma in mice was inconclusive. In addition, in both cases it is unclear why the tumours did not occur in the other species, nor were other mechanisms of action excluded, except for genotoxicity.

In conclusion, classification of transfluthrin in Category 2 is proposed for carcinogenicity.

10.9.3 Conclusion on classification and labelling for carcinogenicity

Classification with carcinogenicity category 2, suspected of causing cancer (H351) is proposed.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The carcinogenicity potential of transfluthrin was tested in two studies performed in animals. There was no information on human data. However, several mechanistic studies relevant for carcinogenicity were available.

Carcinogenicity study in mice

In a carcinogenicity study in mice, 60 animals/sex/dose were exposed for 24 months via food to doses of 0, 10, 100, 1000 ppm transfluthrin (equivalent to 0, 2.1, 19.7, 199.5 and 0, 3.1, 33.3, 279.0 mg/kg bw/day in males and females, respectively). Ten animals/sex/dose were allocated for interim sacrifice after 12 months. In high dose females a statistically significant increase in bw was reported except during the last part of the study. Food and water consumption were not affected. Changes in haematology, clinical chemistry and histopathology revealed the liver as the main target organ in mice in the top dose group and to a lesser extent in the mid dose group. Absolute and relative liver weights were increased ($p < 0.01$) in males and females of the top dose group.

In high dose females statistically significant increased incidences of hepatocellular adenomas (13/50 versus 4/50 in the control) were observed. No increase was observed in high dose males (5/50 versus 5/49 in controls). The number of carcinoma findings on either sex was not increased compared to the control group. It should be noted that the B6C3F1 strain is known to have a high incidence of spontaneously occurring liver tumours. Other pyrethroids have been associated with the occurrence of hepatocellular tumours in rats and/or mice. In several cases, the mode of action was shown to be related to the induction of P450 isozymes through CAR activation. This mechanism is not considered relevant to humans. To address this mechanism for transfluthrin, additional mechanistic studies have been performed and are discussed below.

Further neoplastic lesions observed were an increased incidence in hemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50) and sarcomas of the subcutis (2/50) in high dose females. Incidence of hemangiosarcomas in the spleen were slightly above the historical control data (HCD, 4% vs. 2%) being obtained from 13 two-year studies conducted at the same laboratory. The effect was limited to one sex and was not statistically significant. Since a haemangiosarcoma is vascular in origin, an increased incidence of this tumour type would be expected in other organs if this tumour is treatment related. As there is no increase in the incidence of hemangiosarcomas in any other organ, nor is there an increase in the cumulated incidence (4-0-2-3) of this tumour in all organs, the very slight increase compared to controls is likely to be incidental and unrelated to treatment.

The Harderian gland is not present in humans. Combining hyperplastic and neoplastic (benign and malignant) lesions does not show an increase of the Harderian gland lesions (6-6-6-8) in the present study.

In two high dose females tumours occurred in the skin/subcutis of the flank region, which were classified as "*sarcoma not otherwise specified*". In one animal the single white/beige lesion had a diameter of 3 cm and was of elastic structure. In the other animal, which died on day 715, several nodes were observed, which had varying colours, structure and size up to 3.8 x 2.0 x 1.3 cm being differently composed and consisting in the main area of neoplastic cells with large nuclei and evidence of striation of the cytoplasm. Therefore, a classification as most likely to be a "rhabdomyosarcoma" might have been more appropriate. There is no HCD as both lesions occurred in non-protocol areas. Rhabdomyosarcomas, however, represent a rare lesion with just one case in the RITA Database among 922 female B6C3F1 mice of a study which was performed between August 1995 and September 1997.

Table*: results from the carcinogenicity study in mice*

	0 ppm		10 ppm		100 ppm		1000 ppm	
	m	f	m	f	m	f	m	f
Mortality	9	5	2	2	8	11	8	6
Overall tumour incidence (%)	50	58	42	54	50	56	40	74
Liver								

Eosinophilic focus of cellular alteration	0/50	0/50	0/50	0/50	0/50	1/50	1/50	4/50
Hepatocellular adenoma	5/49	4/50	4/50	2/48	5/50	2/50	5/50	13/50*
Carcinoma	5/49	2/50	8/50	2/48	7/50	4/50	7/50	4/50
Nodule	10/50	7/50	13/50	4/50	13/50	5/50	12/50	15/50
Hypertrophy of periacinal hepatocytes (interim)	0/10	0/10	0/10	0/10	0/10	0/10	10/10	6/10
Hypertrophy of periacinal hepatocytes (final)	0/50	0/50	0/50	0/50	0/50	0/50	38/50#	26/50#

* $p < 0.05$; # $p < 0.001$

Carcinogenicity study in rats

In a 2-year oral guideline study (OECD TG 453, 1981) Wistar (SPF-Cpb) rats received 0, 20, 200, 2000 ppm transfluthrin (purity 95%) in the diet. Seventy rats/sex/group were used and treated for 25 months. The dietary intake was calculated to deliver 0, 1.0, 9.9, 100.4 mg/kg bw/day in males and 0, 1.4, 13.6, 142.1 mg/kg bw/day in females.

The target organs of both sexes were the liver and kidney. The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occurred in both sexes with slight effects being observed in rats exposed to 200 ppm (9.9/13.6 mg/kg bw/day) increasing in a dose dependent manner. Those effects are discussed in more detail in the STOT RE section.

At 2000 ppm, there was an increased incidence of urinary bladder urothelial hyperplasia, as well as an increased incidence of urothelial tumours papilloma and carcinoma in both sexes. The tumour incidences were low (3 papilloma and carcinoma together in both sexes), and no bladder tumours were observed in any of the other dose groups.

In male rats, an increased incidence of hepatocellular adenomas was observed in all doses tested. However, this finding was not significantly different from the control group and there was no dose related response.

A slight increase in incidences of thyroid follicular hyperplasia was observed in the mid and high dose and of cuboidal cells in the thyroid, but the observed effects were not significantly different from the control and there was no increased incidence of tumours in the thyroid. These effects on the thyroid are probably secondary to liver hypertrophy and elevated foreign substance metabolism.

***Table**: results from the carcinogenicity study in rats*

	0 ppm		20 ppm		200 ppm		2000 ppm	
	m	f	m	f	m	f	m	f
Mortality	2	1	3	8	8	6	4	5
Overall tumour incidence (%)	44	64	55	72	56	53	62	50
Liver								

Hepatocellular adenoma	0/59	0/59	3/60	0/60	2/59	0/60	3/58	0/60
Carcinoma	1/59	0/59	0/60	0/60	0/59	0/60	0/58	0/60
Kidney								
Tumour (lipomatous)	0/59	0/59	0/60	1/60	0/59	0/60	2/58	0/60
Carcinoma	0/59	0/59	1/60	0/60	0/59	0/60	0/58	0/60
Urinary bladder								
Papilloma	0/58	0/59	0/59	0/60	0/58	0/60	2/57	1/60
Carcinoma	0/58	0/59	0/59	0/60	0/58	0/60	1/57	2/60

The hypothesized mode of action for transfluthrin induced rat bladder tumours is related to cytotoxicity and regenerative proliferation induced by the primary metabolite tetrafluorobenzoic acid (TFBA), ultimately leading to the production of tumours.

Mechanistic studies on bladder tumours

In several studies the mechanism behind the induction of bladder tumours by transfluthrin was investigated.

- In a four-week study with female rats being exposed to 5000 ppm (327 mg/kg bw/day) of transfluthrin for 1 and 4 weeks the proliferation of urothelial cells was determined with BrdU (5′-bromo-2′-deoxyuridine) labelling. After four weeks a statistically significant increase in absolute kidney weight and a 3.7-fold increase in BrdU labelling index were the main findings. However, there was no correlation between BrdU labelling and the concentration of the TFBA metabolite in the urine (concentrations of TFBA were not provided).
- In a cytotoxicity study of TFBA in the 3T3 cell line and rat bladder epithelial explant cultures, TFBA was not cytotoxic in 3T3 cells with an IC_{50} of >1000 µg/mL. The growth of primary explant cultures of rat bladder epithelial cells was inhibited at 300 µg/mL, explants were dead at 1000 µg/mL.
- Effects of transfluthrin on the bladder epithelium were studied in rats and mice by Scanning Electron Microscopy (SEM). Standard Altromin 1321 diet and acidified Altromin 1321 diet (containing 1.25% NH_4Cl) were administered to rats to evaluate the impact of urinary pH on microcrystal formation. Changes were observed at a dose level of 454 mg/kg bw/day in the 4 weeks Altromin 1321 diet plus 1.25% NH_4Cl group, and at a dose level of 180 mg/kg bw/day in the 13 week group, but not in the 4 week group. At 3 weeks, the concentration of TFBA in the urine was 571 µg/mL in rats in the 180 mg/kg bw/day group, 1065 µg/mL in the 454 mg/kg bw/day + 1.25% NH_4Cl group, and 276 µg/mL in mice at 401 mg/kg bw/day. No increase in hyperplasia or BrdU labelling index was observed in any group. Due to extensive changes also in the control group the mouse results could not be interpreted.
- In a comparative metabolism study with Liverbeads (cryopreserved rat hepatocytes entrapped within an alginate matrix) with cells from rat, mouse, dog and human, the main metabolites in all species were tetrafluorobenzoic (TFB) alcohol and its glucuronide metabolite. After an incubation period of 4 and 24 hours TFBA represented a minor metabolite with rat and mouse cells. After 24 hours the relative percentages were 0.77 and 5.25% using a low concentration of 25 µM transfluthrin

and 1.37 and 4.46% using the high concentration of 250 µM. This acidic metabolite was not detectable with dog or human cells.

- In a comparative cytotoxicity study with TFBA in rat and human urothelial cells the LC_{50} of TFBA was comparable in rat and human cell lines (2.25 vs 2.43 mM).

According to the registrant, these studies suggest that urothelial cytotoxicity and associated regenerative proliferation (caused by high, sustained urinary concentrations of TFBA) as the mechanism of urinary bladder tumour formation in rats exposed for two years to a high dose level of transfluthrin.

In addition, in the carcinogenicity study in mice as well as a 1-year study in dogs no bladder tumours and no histopathological changes of the bladder were reported. Therefore, the registrant considers the bladder tumours in rats not relevant to humans.

A mechanistic study in female rats suggests that transfluthrin induces proliferation of the urothelial cells. However, there was no correlation with the concentration of TFBA. TFBA is cytotoxic in cell lines at high concentrations, but a rat cell line was no more sensitive than a human cell line (LC50 rat 2.25 mM and LC50 human 2.43 mM). The Liverbead study suggests that the species differences in metabolism are minor, but it was observed that the amount of TFBA formed in the Liverbeads was much lower than what was measured in the urine. No increase in hyperplasia or BrdU labelling index was observed in any group and, due to extensive changes also in the control group, the mouse electron microscopy results could not be interpreted.

Actual TFBA levels for the carcinogenicity study were not reported and concentrations of this metabolite in humans are unknown, which means that the comparison remains hypothetical. Although TFBA was determined to be the major metabolite in rats, it is also not proven that TFBA is the only active metabolite, as the studies did not investigate the effects of the other metabolites of transfluthrin. Therefore, the DS considered the evidence for the non-human relevance of bladder tumours in rats to be inconclusive.

Mechanistic studies on liver tumours

In the mouse (females) carcinogenicity study transfluthrin promoted liver tumour development. A number of other pyrethroids have also been associated with the formation of hepatocellular tumours in rats and/or mice when administered at high doses.

Available mutagenicity studies for transfluthrin do not indicate a genotoxic mode of action. Studies on structurally related pyrethroids (e.g., epsilon-metofluthrin) also suggest a non-genotoxic mechanism of action for liver tumour formation. For some pyrethroids, induction of P450 isozymes by constitutive androstane receptor (CAR) activation has been shown to be crucial for the mode of action and induction of liver changes.

Initially, two mechanistic studies were performed to investigate whether transfluthrin is indeed a CAR activator:

The potential of transfluthrin to stimulate cell proliferation and activate several nuclear hormone receptors (Aryl hydrocarbon Receptor (AhR); Constitutive Androstane Receptor (CAR); Pregnane X Receptor (PXR) and Peroxisome Proliferator-Activated Receptor (PPARα)) was investigated in cultured female B6C3FI mouse hepatocytes (Annex I of the CLH report, section 3.9.4.7, study 7). Transfluthrin was not cytotoxic up to the maximum soluble concentration, as indicated by slight precipitation at concentrations of 300 µM and above. Treatment of mouse hepatocytes with 30-1000 µM transfluthrin resulted in a weak induction of mRNA levels of several cytochrome P450 enzymes at the 300 µM concentration. However, the expression of Cyp2b10, which is strongly induced by phenobarbital was only weakly induced by transfluthrin, but not statistically significantly. Conversely, Cyp4a10 and Cyp4a14 were slightly induced by 300 µM transfluthrin but not by phenobarbital. All concentrations inhibited the activity of several liver enzymes (EROD, PROD, BROD, BQ) by a factor of 5 to 100, in contrast to a marked induction by the positive control phenobarbital. No further analyses were performed to explain this finding. Transfluthrin induced an increase in replicative DNA synthesis from 100 µM and above. However, the positive control phenobarbital had no effect on replicative DNA synthesis. Based on this study, no clear statement can be made on the question of whether transfluthrin induces CAR activation.

A second study explored the same parameters in cultured human hepatocytes taken from three different female donors (Annex I to the CLH report section 3.9.4.9, study 9). Treatment of the human hepatocytes resulted again in a dose-dependent inhibition of the liver enzyme BQ (= O-debenzylation, others not tested), which was induced by the positive control WY14643 (PPARα activator). The effects on the expression of mRNA of human CYP enzymes was generally small and showed high variation both between donors and between samples from the same donor. Most notable was CYP3A4, which was induced in one donor but not in the others. There was no statistically significant effect on replicative DNA synthesis.

The most notable difference is the strong inhibition of enzyme activity in mouse liver, which is also observed to a lesser extent in human cells, whereas these enzymes are induced by the positive controls. The implications of this finding are unclear. The gene normally associated with CAR activation, Cyp2b10 in mice, was not activated by transfluthrin.

One effect suggestive of CAR activation is that transfluthrin increases replicative DNA synthesis in mouse hepatocytes but not in human hepatocytes. However, the CAR activator phenobarbital did not induce an increase. A finding that is contrary to what would be expected. It should also be noted that this induction was observed above a concentration of 100 µM. There are no data to show whether this is consistent with exposure of hepatocytes *in vivo*. Unfortunately, there is no study using humanised CAR/PXR knock-out mice to demonstrate specificity for the CAR mechanism of action. As only female mouse hepatocytes were tested, it is unclear whether there are any mechanistic differences between the sexes and between mice and rats. This would have been relevant as it could explain why male mice and rats seem to be less sensitive to the induction of liver tumours by transfluthrin, while they are also sensitive to CAR activators.

Because some results from these *in vitro* experiments were unexpected and inconclusive, two additional experiments were performed. First, in human hepatocytes a slight induction of mRNA for CYP2B6, CYP3A4 and CYP4A11 was observed after 48 hours, but not in mouse hepatocytes. Another study revealed that with a prolonged treatment period of 120 hours slight increases of mRNA for CYP2B10 and CYP3A11 – approximately twofold - were detectable also in murine cells.

An overall evaluation of all *in vitro* studies with mouse and human hepatocytes does not allow a clear-cut conclusion with respect to the mode of the hepatocarcinogenic action of transfluthrin in female mice.

Comments received during consultation

One company-manufacturer disagreed with the classification proposed and commented on the proposed classification as Carc 2. The manufacturer argued that it is not possible to assert human relevance from the mechanistic studies because liver adenomas were only seen in female mice as they received the highest dose and activation of CAR/PPARa genes is lower in humans. In addition, the manufacturer argued that in mouse and human hepatocytes, enzyme levels decreased in an unexpected way and as stated in the CLH report the lack of proliferative response in human hepatocytes is an indication that the mode of action might be irrelevant for humans. Regarding bladder tumours it was argued that the urinary bladder tumours in rats are not relevant for humans, due to a combination of lower exposure, lack of formation of TFBA, and lower sensitivity of the urothelium.

The DS stated that this argumentation is quantitative, diminishing its strength in the context of classification and labelling. There is very limited information on metabolism and excretion of transfluthrin in humans, but in an *in vitro* study low levels of TFBA were found in mouse and rat Liverbeads, whereas TFB alcohol was the main metabolite. As this deviation occurs from *in vivo* studies, the relevance of the outcome in human Liverbeads is questionable as well.

Assessment and comparison with the classification criteria

Classification as Carc. 1A is not justified as no human data are available.

Based on the data available, there is no evidence for a genotoxic mode of action for the effects, as the *in vivo* micronucleus test was negative. However, there is a slightly increased incidence of urinary bladder tumours at the top dose group in both sexes in the rat carcinogenicity study. The mechanistic evidence on the non-human relevance of the bladder tumours in rats is inconclusive.

The possible mode of action for transfluthrin induced bladder tumours is related to cytotoxicity induced by the primary metabolite TFBA, ultimately leading to the production of tumours. In a comparative metabolism study with cells from rat, mouse, dog and human, the main metabolites in all species were TFB alcohol and its glucuronide. TFBA represented a minor metabolite with rat and mouse cells. After 24 hours the relative percentages were 0.77 and 5.25% using a low concentration of 25 µM transfluthrin and 1.37 and 4.46% using the high concentration of 250 µM. This acidic metabolite was not detectable with dog or human cells.

In a comparative cytotoxicity study with TFBA in rat and human urothelial cells the LC_{50} of TFBA was comparable in rat and human cell lines (2.25 vs 2.43 mM).

In high dose females of the mouse carcinogenicity study, increased incidences in hemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50), hepatocellular adenomas (13/50) and sarcomas of the subcutis (2/50) were reported. The low incidences of hemangiosarcomas and sarcomas of the subcutis are considered incidental and not treatment related as the tumours emerged from different tissues. As the Harderian gland is not present in humans, the lesions are not relevant.

All adenomas occurred only in one sex and there was no progression from benign to malignant. The significant increase in benign hepatocellular adenomas was accompanied by increased incidences of hepatocellular hypertrophy in both sexes in the top dose group with increased liver weights. To investigate whether the liver toxicity and hepatocellular adenomas were induced through CAR activation, additional mechanistic studies were conducted with cultured hepatocytes from mouse and human livers. In these studies, transfluthrin inhibited the activity of various liver enzymes in both mouse and human hepatocytes, which is contrary to what would be expected from a CAR activator. The mRNA levels of some P450 enzymes were increased, but the pattern was only partially in agreement with that of CAR activators.

Transfluthrin increases replicative DNA synthesis in mouse hepatocytes but not (statistically significantly) in human hepatocytes. It is unclear whether there is a correspondence between the concentrations at which this induction was observed *in vitro* (⩽ 100 µM) and the *in vivo* concentrations in the liver. Furthermore, this finding alone does not constitute sufficient evidence to conclude that transfluthrin is a CAR/PPARα activator or that hepatocellular adenomas are not relevant to humans.

Based on these mechanistic studies, no definitive conclusion can be drawn about the mode of action of transfluthrin with respect to liver tumours in female mice.

In conclusion, there are tumours observed in two species but with several factors diminishing the strength of evidence. As the mechanistic studies to investigate the human relevance of both the bladder tumours in rats and the hepatocellular adenoma in mice were inconclusive, "no classification" would not be justifiable. Considering the uncertainties, the lack of statistical significance for most tumour types and the type of tumours with significantly increased incidence, classification in Category 2 is considered more appropriate than Category 1B.

Overall, RAC supports the dossier submitter's proposal for **classification of transfluthrin as Carc. 2; H351 (Suspected of causing cancer)**.

10.10 Reproductive toxicity

Not evaluated in this dossier.

10.11 Specific target organ toxicity-single exposure

Table 8: Summary table of animal studies on STOT SE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Route of exposure Relevant information about the study (as applicable)	Results	Reference
Acute oral toxicity in the mouse (OECD 401 (1981))	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5%	Mouse, NMRI (SPF-Han), 5 mice/sex/group Males: 100, 160, 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw Females: 100, 250, 500, 630, 710, 1000 and 5000 mg/kg bw Single exposure, 14 days post exposure period	Mortality: At 250 mg/kg and higher most of the animals died within 24 hours after dosing. Clinical findings: At 160 mg/kg and higher, the symptoms observed point to an effect of the test compound on the nervous system. These symptoms were apparent for a maximum of five days after administration and disappeared rapidly during the observation period. Body weight: No effects on body weight gain were observed. Pathology: Animals that died during the study had some organs that were pale, patchy and/or distended.	Doc. IIIA/Section A6.1.1

Acute dermal toxicity in the mouse OECD 402 (1987) US EPA OPPTS § 870.1200 (1998) Directive 67/548/EEC Annex V, B.3 (1992)	NAK 4455 (transfluthrin), lot/ batch number 816779504, purity 95.8%	Mouse, NMRI:WU, 5 mice/sex/group Concentration: 2000 or 4000 mg/kg Duration of exposure: 24 h Post exposure period: 14 days Dermal exposure	Mortality: Two animals (one each male and female) in the high dose group died during the study. Clinical effects: At 2000 mg/kg bw and above, temporary tremor was observed in both sexes. At 4000 mg/kg bw, motility was affected and temporary convulsions occurred. The symptoms began on day 2 and continued up to day 7 of the study. Body weight: No treatment related effects on body weight gain were observed. Pathology: The male had autolysis, the female discoloured and pale liver, spleen or kidney. No treatment related effects were seen in animals terminated at end of study.	Doc. IIIA/Section A6.1.2
Acute inhalation toxicity in the rat OECD 403 (1981) EC B.2 (1984) FIFRA § 81-3 (1984)	NAK 4455 (transfluthrin), lot/ batch number 250 987 (mixed batch), purity 94.5%	Rat, Bor: WISW (SPF-Cpb), 5 mice/sex/group Nominal concentration: 5000 [mg/m³] Analytical concentration: 513 [mg/m³] MMAD (mass median aerodynamic diameter) 1.44 [µm] ± GSD (geometric standard deviation) 1.42	Clinical effects: slight tremor in exposed female animals resolving within 5 minutes. No other treatment related effects were seen.	Doc. IIIA/Section A6.1.3

Acute Oral Rat neurotoxicity Study This study addresses only the motor activity (open field study) part of OECD TG 424.	BAY U 4619 (transfluthrin), lot/ batch number: 816679301, purity 95.5%	Rat, Wistar (HsdCpb: WU) Number of animals: 6 for combined temperature/catalepsy test, 10 for open field test of psychomotoric activity Dose: 0, 10, 30, 100 mg/kg in a volume of 5 mL/kg	Treatment with transfluthrin is not considered to influence the acute motor activity of rats. However, the study set up shows many deficiencies and the number of parameters tested is very limited. No individual data have been presented. In view of the very limited number of parameters that has been tested in male animals only, this can not be deemed an adequate neurotoxicity study. This study is considered supplementary LO(A)EL: Not established NO(A)EL: Not established	Doc. IIIA/ Section A 6.9
28-Day oral rat study OECD 407 Repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study (1981)	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 95.0%	Rat Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 30 rats/sex/group (except high dose group which had 35/sex/group) Dose: 0, 10, 50, 250 mg/kg bw Study duration: 28 days	Critical effects: transient appearance of tremor, resolving on discontinuation of exposure, seizures in two animals, and the death of 7 animals after previous tremors in the high dose group. These findings are typical of other pyrethroids and were not seen in groups receiving lower doses. Tremors occurred in the early part of the study, and were observed 4-7 h post administration, indicating that this is an acute effect of transfluthrin.	Doc. IIIA/ Section A6.3.1

13-Week inhalation rat study OECD Guideline 413 Subchronic Inhalation Toxicity (1981) US EPA FIFRA § 82-4 Subchronic Inhalation Toxicity (1984)	NAK 4455 (transfluthrin), lot/ batch number 250987, purity 95.0%	Rat, Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 10 rats/ sex/ group (except vehicle control and 1000 mg/m3 groups which had an additional 10 animals/ sex/ group "satellite groups") Dose: Nominal: 0, 40, 250, 1000 Analytical: 0, 4.9, 46.7, 220.2 MMAD 1.1 [µm] + GSD 1.4 [µm] Duration of treatment: 13 weeks	Critical effects: The major finding in this study was post-exposure hyperactivity (resolving the following day) in all animals in the 1000 mg/m^3 group throughout the entire exposure period. In the first week, animals in the high dose group also demonstrated bristling and ungroomed coats and tremor after exposure, resolving by the following day. These signs gradually declined after the 2nd week of exposure. Fluoride levels in bone (males) and teeth (females) were increased in the high dose group. High dose group females had increased polymorphonuclear neutrophils (PMN) in the blood and decreased PMN in the bone marrow. As there does not appear to be a change in absolute white blood cell numbers, this is not judged to be an effect of concern. The combined results of the haematology, clinical chemistry and urinalysis evidenced minor effects which might indicate slight liver and kidney effects. However, these results are neither time nor dose-dependent and are not supported by the histopathological results. Additionally, the effects, while statistically significant, remain largely within normal physiological parameters. LOAEL: 220.2 mg/m3 NOAEL: 46.7 mg/m3	Doc IIIA/Section A 6.4.3

Teratogenicity Study – Rat EPA New and Revised Health Effects Test Guidelines (1984), IRLG Recommended Guidelines (1981), and OECD Guidelines (1981)	Transfluthrin, Batch No. 130187, purity 94.5%	Organism/ species: rat, Charles River Crl:CD BR strain Number of animals: 28 females/group Administration: Oral, by gavage. Doses: 0 (Control), 25, 55 or 120 mg kg bw/day (based on a range finding study) Controls: Vehicle, volume 10 ml/kg.	NOAEL maternal toxicity: 25 mg/kg bw LOAEL maternal toxicity: 55 mg/kg bw/day NOAEL embryotoxicity: 125 mg/kg bw LOAEL embryotoxiicty: > 125 mg/kg bw Critical effects: The critical endpoint for maternal toxicity was tremor occurring after dosing in mid-dose (11%) and high-dose (82%) dams, and death of one high-dose dam.	Doc. IIIA/ Section A6.8.1/01
Teratogenicity Study – Rabbit EPA 83-3 "Teratogenicity Study" (1984), from Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation: Human and Domestic Animals	Transfluthrin, Mixed batch 250 987, 94.5% (27 Oct 1987), 95% (27 April 1988 retest)	Organism/ species: Rabbit, CHBB:Himalayan strain Number of animals: 15 females and males/group Dose: 0 (Control), 15, 50 or 150 mg kg bw/day	NOAEL maternal toxicity: 15 mg/kg bw LOAEL maternal toxicity: 50 mg/kg bw/day NOAEL embryotoxicity: 150 mg/kg bw LOAEL embryotoxicity: >150 mg/kg bw/day Critical effects: The critical endpoint for maternal toxicity was tremor occurring after dosing in one mid-dose and one high-dose dam, followed by death. No treatment-related effect on gestation or foetuses was detected.	Doc. IIIA/ Section A6.8.1/02

Human data on STOT SE

Workers at three facilities have been exposed to transfluthrin during normal production, formulation, and testing of products. Standard workplace protection procedures included use of equipment to prevent dermal and respiratory exposure to fine particulates. Due to the lack of any workplace accidents, no significant acute human exposure is known to have occurred. Routine medical examinations of workers over time have not detected clinical signs related to transfluthrin exposure.

Table 9: Summary table of other studies relevant for STOT SE

No data available.

10.11.1 Short summary and overall relevance of the provided information on specific target organ toxicity – single exposure

Based on the acute toxicity studies available, it can be concluded that transfluthrin caused clinical signs of neurotoxicity including tremors and convulsions after a single exposure. These effects disappeared during the observation period. In the available acute neurotoxicity test these effects were not observed. However, it should be noted that in this test was not deemed to be an adequate neurotoxicity study and is therefore considered supplementary. This was concluded because the study set up shows many deficiencies and the number of parameters tested is very limited. No individual data have been presented. In view of the very limited number of parameters that has been tested in male animals only, this can not be deemed an adequate neurotoxicity study.

Clinical signs of acute neurotoxicity (tremors, seizures apathy, prostration, dyspnoea, and bristling coats) were also observed in repeated dose oral and inhalation studies in rat. In studies in which transfluthrin is administered by gavage (acute oral toxicity studies, 4-week toxicity study, developmental toxicity studies) clinical signs of acute neurotoxicity (tremors, seizures apathy, prostration, dyspnoea, and bristling coats) were observed at doses of 50 mg/kg bw and above.

In the acute oral toxicity study in mice (Doc. IIIA/Section A6.1.1), tremors were observed in males and females dosed 250 mg/kg bw transfluthrin by gavage. Females also showed prostration on the side. Spasmodic tremor, dyspnoea and bristling coasts were observed at dose levels exceeding 250 mg/kg bw. The neurotoxic effects observed in the oral toxicity study in mice were apparent for a maximum of five days after administration. At dose levels of 250 mg/kg bw and above all animals tested (5/5) showed toxicological signs including apathy and tremor. However, no individual animal data were reported. At 250 mg/kg bw/day one male mouse was found death but it remains unknown if this is related to the neurotoxic effects observed. The LD_{50} was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females (see section 10.1).

In the 28-day oral rat study (Doc. IIIA/ Section A6.3.1), tremors were observed 4-7 hours following treatment with 250 mg/kg bw transfluthrin and the effects were not present on the next day. The highest incidence (25/35 males, 22/35 females) of these effects were observed during the first week of the study, but in some animals effects were still observed in week 3 or 4. A total of 7 animals (2/35 males and 5/35 females) died on day 2-3 following administration of 250 mg/kg bw transfluthrin. All these animals, except one female, suffered from tremors. In addition seizures were observed in two females prior to mortality.

In the teratogenicity study in rat (Doc. IIIA/ Section A6.8.1/01), tremors were observed in dams dosed 55 and 125 mg/kg bw with an incidence of 11% and 82%, respectively. These effects were observed within 1 hour after dosing. These effects were transient and resolved in a few hours. In addition, ataxia and salivation was observed in one dam immediately after dosing. One dam out of 28 dosed 125 mg/kg bw was found dead at day 8 and showed tremors on day 6-7. In general, tremors were observed on day 6-15, corresponding with the dosing regimen of days 6-15 post-insemination.

In the teratogenicity study in rabbits (Doc. IIIA/ Section A6.8.1/02), clinical symptoms consistent with pyrethroid toxicity of the central nervous system was observed in one dam out of 15 administered 50 mg/kg bw and one dam out of 15 administered 150 mg/kg bw which was followed by mortality. Mortality was observed on day 18 and 19. However, based on the study report (which is presented in German language) it does not become clear at which day the clinical symptoms started.

For inhalation exposure, neurotoxicity appears to be the critical endpoint. In a 13-week inhalation study (rat), immediately post-dosing hyperactivity, tremors, bristling and ungroomed coat were observed, with a LOAEL of 220.2 mg/m^3. These effects are considered to be acute effects since the neurotoxic effects became apparent in the first week immediately after dosing. The effects resolved by the following day.

The overall LOAEL for acute toxic effects was 50 mg/kg bw based on the neurotoxic effects occurring in the developmental neurotoxicity study in the rabbit.

10.11.2 Comparison with the CLP criteria

Clinical (*e.g.* tremors) evidence of neurotoxicity occurring at dose levels below 300 mg/kg bw/day (oral exposure) / 1000 mg/m^3 / 4hr (inhalation exposure) were found in the acute toxicity studies.

This conclusion is not strengthened by the results from acute neurotoxicity study (oral exposure). However, it should be noted that the acute neurotoxicity study is considered supplementary. According to the guidance value ranges for single-dose exposures laid down in the CLP criteria (Annex I 3.8.2.1.9.3), this effect should be classified as category 1.

Table 3.8.1, defines specific target organ toxicity single exposure, cat. 1 as follows:

'Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following single exposure Substances are classified in Category 1 for specific target organ toxicity (single exposure) on the basis of: a. reliable and good quality evidence from human cases or epidemiological studies; or b. observations from appropriate studies in experimental animals in which significant and/or severe toxic effects of relevance to human health were produced at generally low exposure concentrations. Guidance dose/concentration values are provided below (see 3.8.2.1.9) to be used as part of weight-of-evidence evaluation.'

However, it should be noted that Regulation EC No 1272/2008 (CLP), section 3.8.1 states that:

"Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned."

It should be noted that it is already proposed to classify transfluthrin for acute toxicity Cat. 4 on the basis of the LD_{50} studies with cut-off values of 300-2000 mg/kg bw. STOT SE 1 would thus be a more sensitive endpoint since neurotoxicity findings was observed at dose levels below 300 mg/kg bw/day, with a LOAEL of 50 mg/kg bw. Although clinical effects observed after short term exposure were generally transient, without histopathological correlate and were not observed in a (supplemental) neurotoxicity study, clinical effects were observed co-occurring with mortality in the acute toxicity study (Doc. IIIA/Section A6.1.1), 28-day oral rat study (Doc. IIIA/ Section A6.3.1), the teratogenicity study in rat (Doc. IIIA/ Section A6.8.1/01) and the teratogenicity study in rabbits (Doc. IIIA/ Section A6.8.1/02). The observed mortality is presumably caused by the neurotoxic effects. Transfluthrin belongs to the class of pyrethroids which are known to exert neurotoxic effects. According to the CLP criteria mortalities observed within 72 hours after the first treatment can be considered an acute effect. Mortality seems to be attributed to multiple exposures in the teratogenicity study in rabbits but was observed within 1 day in the acute toxicity (Doc. IIIA/Section A6.1.1) study and 2-3 days following dosing in both the 28-day oral rat study (Doc. IIIA/ Section A6.3.1) and the teratogenicity study in rat (Doc. IIIA/ Section A6.8.1/01).
Based on the available data it was demonstrated that the neurotoxic effects can lead to mortality at dose levels that are below the classification criteria for acute toxicity Cat. 4. (300-2000 mg/kg bw).

In addition, neurotoxic effects were observed after inhalation exposure at a dose of 220.2 mg/m^3 in a 13 week study. The inhalation guidance value for classification for STOT SE 1 is 1.0 mg/l/4h (1000 mg/m^3/4hr). Because the study is sub-chronic, a direct comparison is hampered as animals were exposed for a longer period than four hours, but it was reported that neurotoxic effects occurred immediately after dosing. Hence, these effects are considered relevant for classification.

As neurotoxic effects consistently occur directly after dosing at dose levels below the limit values and also below the cut-off value for acute tox 4, it is proposed to classify transfluthrin for STOT SE (nervous system) Cat 1. As relevance for inhalation cannot be excluded, no specific route is proposed.

The assignment of STOT SE 3 can be done independently of STOT RE 1 and 2.

Table 3.8.1, defines specific target organ toxicity single exposure, cat. 3 as follows:

Transient target organ effects. This category only includes narcotic effects and respiratory tract irritation. These are target organ effects for which a substance does not meet the criteria to be classified in Categories 1 or 2 indicated above. These are effects which adversely alter human function for a short duration after exposure and from which humans may recover in a reasonable period without leaving significant alteration of structure or function. Substances are classified specifically for these effects as laid down in 3.8.2.2.

For narcotic effects the following criteria are defined:

(a) central nervous system depression including narcotic effects in humans such as drowsiness, narcosis, reduced alertness, loss of reflexes, lack of coordination, and vertigo are included. These effects can also be manifested as severe headache or nausea, and can lead to reduced judgment, dizziness, irritability, fatigue, impaired memory function, deficits in perception and coordination, reaction time, or sleepiness.

(b) narcotic effects observed in animal studies may include lethargy, lack of coordination, loss of righting reflex, and ataxia. If these effects are not transient in nature, then they shall be considered to support classification for Category 1 or 2 specific target organ toxicity single exposure.

No human data are available. Although several neurotoxic effects were observed (tremors, seizures, apathy, prostration, dyspnoea, and bristling coats) in the available animal studies, transfluthrin did not cause any narcotic effects apart from ataxia observed in one dam out of 28 tested in the teratogenicity study in rat. Therefore, classification with STOT SE 3 is not justified.

10.11.3 Conclusion on classification and labelling for STOT SE

Classification as STOT SE Category 1 (H370, causes damage to the nervous system) is proposed.

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

Summary of the Dossier Submitter's proposal

To evaluate the specific target organ toxicity after single exposure the DS listed eight studies in rats, mice and rabbits.

In the acute oral toxicity study in mice, tremors were observed in both sexes dosed with 250 mg/kg bw transfluthrin by gavage. Spasmodic tremor, dyspnea and bristling coat were observed at dose levels above 250 mg/kg bw. Neurotoxic effects were apparent for a maximum of five days after administration. At dose levels of 250 mg/kg bw and above all animals showed toxicological signs including apathy and tremor. The LD_{50} was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females.

One acute oral rat neurotoxicity study did not show any tremors or convulsion after exposure to transfluthrin at doses up to 100 mg/kg bw. The DS noted that this test was not deemed to be an adequate neurotoxicity study and considered it as supplementary. This conclusion was made due to the deficiencies of the study set up and the limited number of tested parameters.

In one 28-day oral rat study tremors were observed 4-7 hours following treatment with 250 mg/kg bw transfluthrin. The DS described that these effects were not present on the next day. A total of seven rats (2 males, 5 females) died on days 2-3 following administration of 250 mg/kg bw transfluthrin. All these animals, except one female, suffered from tremors.

The DS described two teratogenicity studies. In the study in rats, tremors were observed at dose levels of 55 and 125 mg/kg bw with an incidence of 11% and 82%. The effects were observed within one hour after dosing. In rabbits, clinical symptoms of the central nervous system were observed in one of 15 dams administered 50 mg/kg bw and in one of 15 dams administered 150 mg/kg bw which was followed by death of the animal.

Hyperactivity, tremors, bristling and ungroomed coat, appearing immediately after dosing, were observed in one 13-week inhalation study in rats. The LOAEL was 220.2 mg/m^3.

The DS concluded that neurotoxic effects consistently occurred directly after dosing at dose levels below the limit values for STOT SE and below the cut-off value for acute tox 4. Consequently, they proposed to classify transfluthrin for STOT SE (nervous system) category 1. As the relation with the inhalation route cannot be excluded, no specific route is proposed.

Comments received during consultation

One MSCA supported classification as STOT SE 1.

One Company-Manufacturer commented and disagreed with classification of transfluthrin as STOT SE 1. They argued that this would lead to over classification for single exposure toxicity and would not follow the recommendations of the CLP guidance to avoid double classification. Acute Toxicity 4 classification is already proposed for oral toxicity based on effects to the nervous system. The Company-Manufacturer concluded that STOT SE category 2 classification would be more appropriate.

The DS disagreed with this conclusion. The potency of the substance is an important factor in determining the classification category for STOT SE. The effects observed in the studies included in the CLH report, as well as in the new study, occur below the guidance threshold value for STOT SE 1 of 300 mg/kg bw. The argument of double classification for mortality would have been warranted if transfluthrin would have been classified as Acute Tox. Cat. 3 (corresponding to acute toxicity estimates between 50 and 300 mg/kg bw). The incidence of mortality after single exposure at doses below 300 mg/kg bw is too low to warrant classification for acute toxicity. For this reason, category 4 was proposed for Acute oral toxicity. As neurotoxicity is a more sensitive endpoint than mortality, the DS remained of the opinion that classification as STOT SE 1 is warranted.

Assessment and comparison with the classification criteria

Table: *summary table of animal data*

Method, guideline, deviations if any, species, strain, sex, no/group	Route of exposure Relevant information about the study (as applicable)	Results	Reference
Acute oral toxicity in the mouse (OECD 401 (1981))	Mouse, NMRI (SPF-Han), 5 mice/sex/group Males: 100, 160, 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw Females: 100, 250, 500, 630, 710, 1000 and 5000 mg/kg bw Single exposure, 14 days post exposure period	Mortality: At 250 mg/kg bw and higher most of the animals died within 24 hours after dosing. At 160 mg/kg bw and higher, effects on the nervous system were observed. These symptoms were apparent for a maximum of five days after administration and disappeared rapidly during the observation period.	Doc. IIIA/Section A6.1.1
Acute inhalation toxicity in the rat OECD 403 (1981) EC B.2 (1984) FIFRA § 81-3 (1984)	Rat, Bor: WISW (SPF-Cpb), 5 mice/sex/group Nominal concentration: 5000 [mg/m³] Analytical concentration: 513 [mg/m³] MMAD (mass median aerodynamic diameter) 1.44 [µm] ± GSD (geometric standard deviation) 1.42	Clinical effects: slight tremor in exposed female animals resolving within 5 minutes. No other treatment related effects were observed.	Doc. IIIA/Section A6.1.3

Acute oral rat neurotoxicity study This study addresses only the motor activity (open field study) part of OECD TG 424	Rat, Wistar (HsdCpb: WU) Number of animals: 6 for combined temperature/catalepsy test, 10 for open field test of psychomotoric activity Dose: 0, 10, 30, 100 mg/kg bw in a volume of 5 mL/kg bw	Treatment with transfluthrin is not considered to influence the acute motor activity of rats. In view of the very limited number of parameters that has been tested in male animals only, this cannot be deemed an adequate neurotoxicity study. This study is considered supplementary LO(A)EL: Not established NO(A)EL: Not established	Doc. IIIA/ Section A 6.9
28-Day oral rat study OECD 407 Repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study (1981)	Rat Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 30 rats/sex/group (except high dose group which had 35/sex/group) Dose: 0, 10, 50, 250 mg/kg bw/day Study duration: 28 days	Tremors occurred in the early part of the study and were observed 4-7 h post administration, indicating that this is an acute effect of transfluthrin.	Doc. IIIA/ Section A6.3.1
13-Week inhalation rat study OECD TG 413 Subchronic Inhalation Toxicity (1981) US EPA FIFRA § 82-4 Subchronic Inhalation Toxicity (1984)	Rat, Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 10 rats/sex/group (except vehicle control and 1000 mg/m^3 groups which had an additional 10 animals/sex/group "satellite groups") Dose: Nominal: 0, 40, 250, 1000 [mg/m^3] Analytical: 0, 4.9, 46.7, 220.2 [mg/m^3] MMAD 1.1 [µm] + GSD 1.4 [µm] Duration of treatment: 13 weeks	Post-exposure hyperactivity (resolving the following day) in all animals in the 1000 mg/m^3 group throughout the entire exposure period. LOAEL: 220.2 mg/m^3 NOAEL: 46.7 mg/m^3	Doc IIIA/Section A 6.4.3

Teratogenicity Study – Rat EPA New and Revised Health Effects Test Guidelines (1984), IRLG Recommended Guidelines (1981), and OECD TGs (1981)	Organism/species: rat, Charles River Crl:CD BR strain Number of animals: 28 females/group Administration: Oral, by gavage. Doses: 0 (Control), 25, 55 or 125 mg kg bw/day (based on a range finding study) Controls: Vehicle, volume 10 mL/kg bw.	NOAEL maternal toxicity: 25 mg/kg bw/day LOAEL maternal toxicity: 55 mg/kg bw/day NOAEL embryotoxicity: 125 mg/kg bw/day LOAEL embryotoxicity: > 125 mg/kg bw/day Tremor occurred after dosing in mid-dose (11%) and high-dose (82%) dams, and death of one high-dose dam.	Doc. IIIA/ Section A6.8.1/01
Teratogenicity Study – Rabbit EPA 83-3 "Teratogenicity Study" (1984),	Organism/ species: Rabbit, CHBB: Himalayan strain Number of animals: 15 dams /group Dose: 0 (Control), 15, 50 or 150 mg kg bw/day	NOAEL maternal toxicity: 15 mg/kg bw LOAEL maternal toxicity: 50 mg/kg bw/day NOAEL embryotoxicity: 150 mg/kg bw LOAEL embryotoxicity: >150 mg/kg bw/day Tremor occurred after dosing in one mid-dose and one high-dose dam, followed by death. No treatment-related effect on gestation or foetuses was detected.	Doc. IIIA/ Section A6.8.1/02

Tremors were observed in the acute oral toxicity study in male and female mice at 250 mg/kg bw transfluthrin by gavage (Doc. IIIA/Section A6.1.1). Spasmodic tremor, dyspnoea and ruffled fur were observed at dose levels exceeding 250 mg/kg bw. These effects were apparent for up to five days after administration. The LD_{50} was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females.

Clinical signs of acute neurotoxicity (tremors, seizures) as well as apathy, prostration, dyspnoea, and ruffled fur were also observed in repeated dose oral (Doc. IIIA/ Section A6.3.1) and inhalation (Doc IIIA/Section A 6.4.3) studies in rats. In studies in which transfluthrin was administered by gavage (acute oral toxicity studies, 4-week toxicity study, developmental toxicity studies) clinical signs of acute neurotoxicity were observed at doses of 50 mg/kg bw and above.

In the 28-day oral rat study (Doc. IIIA/Section A6.3.1), tremors were observed 4-7 hours after treatment with 250 mg/kg bw transfluthrin and the effects were no longer present the next day. The highest incidence (25/35 males, 22/35 females) of these effects was observed in the first week of the study, but in some animals the effects were still observed after 3 or 4 weeks. A total of 7 animals (2/35 males and 5/35 females) died on day 2-3 after administration of 250 mg/kg bw transfluthrin. These animals, except one female, suffered from tremors. In addition, seizures were observed in two females before death.

In the teratogenicity study in the rat (Doc. IIIA/Section A6.8.1/01), tremors were observed in dams administered 55 and 125 mg/kg bw/day with an incidence of 11% and

82%, respectively. These effects were observed within 1 hour of administration but resolved within a few hours.

In the teratogenicity study in rabbits (Doc. IIIA/Section A6.8.1/02), clinical signs consistent with central nervous system pyrethroid toxicity were observed in one of 15 dams administered 50 mg/kg bw/day and one of 15 dams administered 150 mg/kg bw/day, followed by mortality. It is not clear from the study report on which day the clinical symptoms started.

For inhalation exposure, neurotoxicity appears to be the critical endpoint. In a 13-week inhalation study (Doc IIIA/Section A 6.4.3), hyperactivity, tremors, ruffled fur were observed immediately after dosing, with a LOAEL of 220.2 mg/m^3. These effects, resolved by the following day and gradually declining after the 2nd week of exposure, are hence considered to be acute.

Conclusion

It is noted that the Guidance on the Application of the CLP Criteria, 2017, hereinafter referred as "CLP Guidance", section 3.8.1 states that:

"*Acute toxicity refers to lethality and STOT-SE to non-lethal effects. However, care should be taken not to assign both classes for the same toxic effect, essentially giving a "double classification", even where the criteria for both classes are fulfilled. In such case the most appropriate class should be assigned*."

Transfluthrin is a member of the pyrethroids, a chemical class of compounds which are known to exert neurotoxic effects. The DS proposed to classify transfluthrin for Acute Tox. 4, based on studies with LD_{50} values between 300-2000 mg/kg bw. As neurotoxic effects occurred at dose levels below 300 mg/kg bw with a LOAEL of 50 mg/kg bw a classification as STOT SE 1 would be more appropriate for this dose levels. Although clinical effects observed after short term exposure were generally transient, with no histopathological correlation and not observed in a (supplemental) neurotoxicity study, they co-occurred with mortality in the acute toxicity study, 28-day oral rat study and the teratogenicity studies in rats and rabbits. According to the CLP criteria, mortalities observed within 72 hours after the first treatment can be considered an acute effect. However, mortality occurred after multiple exposures in the teratogenicity study in rabbits and rats and the 28-day oral rat study, even if it was also observed within 1 day after exposure in the acute toxicity study and following the first dose in the teratogenicity study in rat. Most importantly, clinical signs of acute neurotoxicity were evident in all the studies provided and at dose levels below those required for classification as Acute Tox 4.

In addition, neurotoxic effects were observed after inhalation exposure at a dose of 220.2 mg/m^3 in a 13-week study. The inhalation guideline value for classification for STOT SE 1 is 1.0 mg/L/4 h (1000 mg/m^3/4 h). As this is a subchronic study, a direct comparison is difficult as animals were exposed for longer than four hours, but neurotoxic effects were reported to occur immediately after dosing. Therefore, these effects are considered relevant for classification.

No data are available in humans.

Transfluthrin did not cause any narcotic effects. A STOT SE 3 classification is therefore not justified.

As neurotoxic effects consistently occurred directly after dosing at dose levels below the guidance values and also below the cut-off value for Acute Tox. 4, RAC agrees with the DS to **classify transfluthrin as STOT SE 1; H370 (causes damage to the nervous system)**. As relevance for inhalation cannot be excluded, no specific route is proposed.

10.12 Specific target organ toxicity-repeated exposure

Table 18: Summary table of animal studies on STOT RE

Method, guideline, deviations if any, species, strain, sex, no/group	Test substance, route of exposure, dose levels, duration of exposure	Results	Reference
28-Day oral rat study OECD 407 Repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study (1981)	Test substance: NAK 4455 (transfluthrin), lot/ batch number 130187, purity 95.0% Organism/ species: Rat Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 30 rats/sex/group (except high dose group which had 35/sex/group) Dose: 0, 10, 50 250 mg/kg bw Study duration: 28 days	LO(A)EL: 250 mg/kg bw/day on the basis of tremors, seizures and mortality, and increased relative liver weight (17-20%) NO(A)EL: 50 mg/kg bw/day Critical effects: transient appearance of tremor, resolving on discontinuation of exposure, seizures in two animals, and the death of 7 animals after previous tremors in the high dose group. These findings are typical of other pyrethroids and were not seen in groups receiving lower doses. Tremors occurred in the early part of the study, and were observed 4-7 h post administration, indicating that this is an acute effect of transfluthrin. More subtle changes included a transient decrease in clotting time in males in the high dose group, a transient increase in liver weight in males and females in the top dose group and liver enzyme induction (O-demethylase followed by N-demethylase) in males in the high dose group. Kidneys in males and females were also transiently increased in weight. The urinalysis did not reveal any particular concerns, however, after sedimentation urine of both sexes was found to contain epithelial cells.	Doc. IIIA/ Section A6.3.1

21-Day dermal rabbit study OECD 410 Repeated Dose Dermal Toxicity Rodent: 21/28-day Study (1981) EPA FIFRA § 82-2 Repeated Dose Dermal Toxicity: 21 day study (1982)	<u>Test substance:</u> NAK 4455 techn. (transfluthrin), lot/ batch number 250987, purity 95.0% <u>Organism/ species:</u> rabbit, HC:NZW (New Zealand White) <u>Number of animals:</u> 5 rabbits/sex/group (except control and high dose group which had 10/sex/group) <u>Dose:</u> 0, 20, 200, 1000 mg/kg bw <u>Study duration:</u> 21 days	<u>NOAEL systemic:</u> 1000 mg/kg bw/day (highest dose tested). <u>NOAEL local:</u> 20 mg/kg bw/day, on the basis of redness, scaling, encrustation, swelling, red patches, increased skin fold thickness, thickening of the epidermis, and hyperkeratosis. <u>Critical effects:</u> The principal finding was reddening of the skin. No systemic effects were noted. At 1000 mg/kg bw/day, only minor localised effects at the skin application site were found.	Doc. IIIA/ Section A6.3.2
18-Week oral rat study US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)	<u>Test substance:</u> NAK 4455 (transfluthrin), lot/ batch number 130187, purity 95.0% <u>Organism/ species:</u> Rat, Bor:WISW (SPF-Cpb) (Wistar) <u>Number of animals:</u> rats/ sex/ group (except control and 5000 ppm groups which had an additional 10 animals/ sex/ group “satellite groups”) <u>Dose:</u> Males: 0, 0.8, 3.5, 37.5 and 384.1 (397.2 in satellite group) mg/kg bw Females: 0, 0.9, 4.4, 47.3 and 515.4 (487.5 in satellite group) mg/kg bw <u>Study duration:</u> 18 weeks	<u>LO(A)EL:</u> 500 ppm (37.5 mg/kg bw/day), on the basis of increased liver weight and centrilobular hypertrophy (both sexes), increased relative kidney weight (males only) and effects on clinical chemistry parameters. <u>NO(A)EL:</u> 50 ppm (3.5 mg/kg bw/day) <u>Critical effects:</u> main observations liver: increased relative liver weight in both sexes at 500 ppm and 5000 ppm (14% and 44% in males, 17% and 28% in females), enlarged livers in males at 500 ppm and 5000 ppm (3/10 and 2/10 vs. 0/10 in controls), and centrilobular hypertrophy in both sexes at 500 ppm and 5000 ppm (8/10 and 10/10 in males vs. 0/10 in controls, 4/10 and 9/10 in females vs. 0/9 in controls). Further liver enzyme activities were increased and some clinical chemistry parameters altered. Kidney and thyroid: in male rats at 500 and 5000 ppm the relative kidney weight was increased (11% and 14%) and thyroid hypertrophy is noted (10/10 and 10/10 vs. 0/10 in controls).	Doc IIIA/Section A 6.4.1/01

3-month oral dog study OECD Guidelines 409 (1981) US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)	Test substance: NAK 4455 (transfluthrin), lot/ batch number 250987, purity 94.5% Organism/ species: Dog, Beagle Number of animals: 4 dogs/ sex/ group Study duration: 3 months	LO(A)EL: 2500 ppm both sexes (equivalent to 93 mg/kg bw/d) NO(A)EL: 350 ppm both sexes (equivalent to 14 mg/kg bw/d) Critical effects: In the higher dose groups, liver weights were increased, liver enzymes were induced and centrilobular hypertrophy was observed, although no lipid vacuolation was seen, suggesting an adaptive response rather than any kind of damage. Additionally, lipids, cholesterol and triglyceride levels were all increased. Increased thyroid weights and decreased levels of thyroid hormones were seen in female animals. This may be a secondary result of altered liver physiology.	Doc IIIA/Section A 6.4.1/02
4-Week inhalation rat study OECD Guideline 412 Subacute Inhalation Toxicity (1983)	NAK 4455 (transfluthrin), lot/ batch number 130187, purity 94.5% Rat, Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 10 rats/ sex/ group Dose: Nominal: 0, 15, 60, 250, 1000 Analytical: 0, 1.5, 6.6, 36.6, 168.1 MMAD 1.5 [µm] + GSD 1.1 [µm] Duration of treatment: 4 weeks	Critical effects: reduced motility, bristling and ungroomed coats in all animals in the 1000 mg/m^3 group. It should be noted that it remains unclear when these effects were first observed and if/when effects resolved. LOAEL: 168.1 mg/m^3 (approximately equivalent to 60 mg/kg bw/d) NOAEL: 36.6 mg/m^3 (approximately equivalent to 13 mg/kg bw/d)	Study 6.3.3 Study not included in the CAR since it was considered a 'non-key study'.

13-Week inhalation rat study OECD Guideline 413 Subchronic Inhalation Toxicity (1981) US EPA FIFRA § 82-4 Subchronic Inhalation Toxicity (1984)	Test substance: NAK 4455 (transfluthrin), lot/ batch number 250987, purity 95.0% Organism/ species: Rat, Bor:WISW (SPF-Cpb) (Wistar) Number of animals: 10 rats/ sex/ group (except vehicle control and 1000 mg/m3 groups which had an additional 10 animals/ sex/ group “satellite groups”) Dose: Nominal: 0, 40, 250, 1000 Analytical: 0, 4.9, 46.7, 220.2 MMAD 1.1 [µm] + GSD 1.4 [µm] Duration of treatment: 13 weeks	LO(A)EL: 220.2 mg/m^3 (approximately equivalent to 79 mg/kg bw/d) NO(A)EL: 46.7 mg/m^3 (approximately equivalent to 17 mg/kg bw/d) Critical effects: The major finding in this study was post-exposure hyperactivity (resolving the following day) in all animals in the 1000 mg/m^3 group throughout the entire exposure period. In the first week, animals in the high dose group also demonstrated bristling and ungroomed coats and tremor after exposure, resolving by the following day. These signs gradually declined after the 2nd week of exposure. Fluoride levels in bone (males) and teeth (females) were increased in the high dose group. High dose group females had increased polymorphonuclear neutrophils (PMN) in the blood and decreased PMN in the bone marrow. As there does not appear to be a change in absolute white blood cell numbers, this is not judged to be an effect of concern. The combined results of the haematology, clinical chemistry and urinalysis evidenced minor effects which might indicate slight liver and kidney effects. However, these results are neither time nor dose-dependent and are not supported by the histopathological results. Additionally, the effects, while statistically significant, remain largely within normal physiological parameters.	Doc IIIA/Section A 6.4.3
2-year oral rat study	Test substance: NAK 4455 (transfluthrin), Mixed batch no 130187, from 10.11.87 250987, purity 95.0% (130187), 94.5% (250987) Organism/ species: Rat, Wistar; Bor:WISW (SPF-Cpb) Number of animals: 70 rats/sex/group Study duration: 25 months Dose: Males: 0, 1.0, 9.9, 100.4 mg/kg bw-day Females: 0, 1.4, 13.6, 142.1 mg/kg bw-day	LOAEL: 200 ppm, equal to 9.9 mg/kg bw/day. Based on the effects observed in the kidney (glomerulonephrosis, pigment deposition, increased absolute and relative weight) NOAEL: 20 ppm, equal to 1.0 mg/kg bw/day Critical effects: Transfluthrin induces glomerulonephrosis at 200 ppm and higher. The urinary bladder urothelial hyperplasia, thyroid follicular hyperplasia and increased cuboidal cells (m+f) and urinary bladder tumours (papilloma and carcinoma), observed at 2000 ppm, are considered to be treatment-related. The tumours in thyroid and liver are considered not related to treatment.	Doc IIIA/Section A 6.5/01, Doc IIIA/Section A 6.7/01

2-year oral mouse study OECD 451 (1981) US EPA FIFRA § 82-2 (1984)	Test sunstance: NAK 4455 (transfluthrin), Mixed batch no 250987, purity 94.5- 95% Organism/ species: Mice, B6C3F1 Number of animals: 60 rats/sex/group (+ extra 10 rats/sex/group for 0 and 1000 ppm groups) Dose: Males: 0, 2.1, 19.7, 199.5 mg/kg bw-day Females: 0, 3.1, 33.3, 279.0 mg/kg bw-day Study duration: 24 months	LOAEL: 100 ppm, equal to 19.7 mg/kg bw/day, based on the observed changes in haematology and clinical chemistry NOAEL: 10 ppm, equal to 2.1 mg/kg bw/day Critical effects: in the females at 1000 ppm there may be a treatment-related increase in haemangiosarcomas in the spleen, adenomas in the Harderian gland and sarcomas of the subcutis. The incidences of these neoplastic lesions are above the historical control range and are considered possibly related to treatment.	Doc IIIA/Section A 6.5/02, Doc IIIA/Section A 6.7/02
1 year oral dog study OECD Guidelines 452 (1981)	Test substance: NAK 4455 (transfluthrin), lot/ batch number 250987, purity 95.1% Organism/ species: Dog, Beagle Number of animals: 4 dogs/sex/group Dose: 0, 1, 10, 100 mg/kg bw/day Study duration: 1 year	LOAEL: 3000 ppm, equivalent to 100 mg/kg bw/day on the basis of liver effects NOAEL: 300 ppm, equivalent to 10 mg/kg bw/day Critical effects: Effects on the liver at the top dose. In all treated animals ALAT, a non-specific marker of liver damage was increased, as was AP and GLDH and cholesterol. However, these changes do not appear to be dose or time responsive and do not appear to reflect a toxicologically adverse effect except at the top dose (3000 ppm). Additionally, N-demethylase levels were elevated in top dose animals and bilirubin levels were decreased supporting the indication of liver effects. All treated animals have reduced liver triglyceride levels, there is no indication of dose-response, and given the normal variability in liver triglycerides and the absence of gross or histopathological changes in the liver, this effect does not have toxicological significance. In the higher dose groups, relative and absolute liver weights were increased; no histopathological change was in seen in the liver or any other organ in treated animals.	Doc IIIA/Section A 6.5/03

1 year oral dog study OECD Guidelines 452 (1981)	Test substance: NAK 4455 (transfluthrin), lot/ batch number 250987, purity 94.7% Organism/ species: Dog, Beagle Number of animals: 4 dogs/sex/group Dose: 0, 0.25 mg/kg bw/day Study duration: 1 year (53 weeks)	LOAEL: - NOAEL: 10 ppm, equivalent to 0.25 mg/kg bw/day (highest dose tested) Critical effects: no treatment related effects were observed.	Study 6.5/04 Study not included in the CAR since it was considered a 'non-key study'.
Teratogenicity Study – Rat EPA New and Revised Health Effects Test Guidelines (1984), IRLG Recommended Guidelines (1981), and OECD Guidelines (1981)	Test substance: Transfluthrin, Batch No. 130187, purity 94.5% Organism/ species: rat, Charles River Crl:CD BR strain Number of animals: 28 females/group Administration: Oral, by gavage. Doses: 0 (Control), 25, 55 or 120 mg kg bw/day (based on a range finding study) Controls: Vehicle, volume 10 ml/kg.	NOAEL maternal toxicity: 25 mg/kg bw, based on post-dosing tremor in pregnant females. NOAEL embryotoxicity: 125 mg/kg bw, based on the absence of findings at the highest dose tested. Critical effects: The critical endpoint for maternal toxicity was tremor occurring after dosing in mid-dose (11%) and high-dose (82%) dams, and death of one high-dose dam. No treatment-related effect on gestation or foetuses was detected.	Doc. IIIA/ Section A6.8.1/01
Teratogenicity Study – Rabbit EPA 83-3 "Teratogenicity Study" (1984), from Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation: Human and Domestic Animals	Test substance: transfluthrin, Mixed batch 250 987, 94.5% (27 Oct 1987), 95% (27 April 1988 retest) Organism/ species: Rabbit, CHBB:Himalayan strain Number of animals: 15 females and males/group Dose: 0 (Control), 15, 50 or 150 mg kg bw/day	NOAEL maternal toxicity: 15 mg/kg bw, based on death of one dam in both the 50 and 150 mg/kg bw dosed groups, preceded by clinical symptoms consistent with pyrethroid toxicity of the central nervous. NOAEL embryotoxicity: 150 mg/kg bw, based on the absence of findings at the highest dose tested. Critical effects: The critical endpoint for maternal toxicity was tremor occurring after dosing in one mid-dose and one high-dose dam, followed by death. No treatment-related effect on gestation or foetuses was detected.	Doc. IIIA/ Section A6.8.1/02

Developmental neurotoxicity Study (U.S. EPA, OPPTS 870.6300 OECD TG 426 (draft) Health Canada PMRA DACO No. 4.5.14)	Test substance: Transfluthrin technical, lot. Batch number EATFTJ005, purity 99.1% Organism/ species: Rat, Wistar crl:WI(Han) Number of animals: 30 females per group Control: concurrent control group given control diet Duration of treatment: Daily, starting on Gestation Day (GD) 6 and continuing for the dams and offspring until lactation day (LD) 21 Dose: 0, 42.1, 161 and 534 mg/kg bw/day	NOAEL: Maternal: 534 mg/kg bw/day Offspring: 161 mg/kg bw/day Critical effects: Maternal 500, 2000 and 7000 ppm: there were no treatment-related findings during gestation or lactation. 7000 ppm: Bodyweight gain during gestation was reduced 10% compared to controls and bodyweight was statistically reduced (6% maximum) on LD0, 4 and 7. These differences from control were ascribed to palatability and were not considered an adverse effect. Offspring 500 and 2000 ppm: there were no treatment-related findings. 7000 ppm: bodyweight was statistically decreased (9%) in females on PND 11. Bodyweight gain was statistically decreased on PND 4-11 in females and combined males and females (11% and 10%, respectively). Also, bodyweight gain was statistically decreased 8-9% on PND 4-21 in males and females. These effects at the highest dose level were associated with decreased bodyweight in the dams, compared to controls.	Doc. IIIA Section A 6.9

Table 19: Summary table of human data on STOT RE

No data available.

Table 20: Summary table of other studies relevant for STOT RE

No data available.

10.12.1 Short summary and overall relevance of the provided information on specific target organ toxicity – repeated exposure

The main targets for repeated dose toxicity appear to be the liver and the kidney.

The effects on the liver and kidney were observed in short term and long term toxicity studies in rat and dog.

In the 18 week rat study (Doc IIIA/Section A 6.4.1/01), centrilobular hypertrophy (liver) (minimal or moderate) was seen in most animals in the high dose group (regular and satellite); minimal centrilobular hypertrophy was seen in 8/10 males and 4/10 females in the 500 ppm group (equal to 37.5 mg/kg bw and 47.3 mg/kg bw for males and females, respectively). Statistically significant increased levels of all liver enzymes were seen in both sexes in the 5000 ppm group (with the exception of P450 in the female rat, equal to 384.1 mg/kg bw and 515.4 mg/kg bw/day for males and females, respectively). In the higher dose groups, liver weights were significantly increased (>10%) and enlarged liver were observed in males only (3/10 and 2/10 at dose levels of 500 and 5000 ppm, respectively). In general, these effects are considered adaptive in nature, rather than an adverse effect on the liver.

Liver damage was also observed in the mouse carcinogenicity study, with a LOAEL of 19.7/33.3 mg/kg bw/d. However, no effects were observed below the guidance value for classification.

Absolute and relative kidney weights were increased in males in the 500 and 5000 (regular and satellite) dose groups. Moreover, in the same groups, a slightly increased number of animals with yellow granular deposits in the epithelial cells of basophilic cortical tubules was observed. However, as this effect was also observed in control animals, a relation to treatment is doubtful.

Histopathological findings in the 3-month dog study revealed centrilobular hypertrophy (liver) in all animals in the high dose group (Doc IIIA/Section A 6.4.1/02). Minimal single-cell necrosis was noted in the liver in one female in the high dose group. In the higher dose groups, liver weights were increased (>10% based on absolute weight), liver enzymes were induced and centrilobular hypertrophy was observed, although no lipid vacuolation was seen. Additionally, lipids, cholesterol and triglyceride levels were all increased.

In the 2-year rat study (Doc IIIA/Section A 6.5/01), seven of ten males in the 2000 ppm (equal to 110.4 mg/kg bw and 142.1 mg/kg bw for males and females, respectively) dose group were found to have rough kidney surfaces. Absolute and relative kidney and liver weights were increased in males and females in the high dose groups. At the 12-month interim autopsy, absolute kidney weight in females in the 200 ppm group (equal to 9.9 mg/kg bw and 13.6 mg/kg bw for males and females, respectively) was elevated. Glomerulonephrosis was seen in males in the 200 and 2000 ppm dose groups, yellow-brown pigment deposits were seen in the tubular epithelial cells and interstitial tissue of the kidneys of both male and female animals in the 200 and 2000 ppm dose groups in an apparently dose-dependent manner. At the 24-month final autopsy absolute kidney weight was also increased in males and females in the 200 ppm dose group, at was relative kidney weight in males in the 200 ppm group and relative liver weight in all treated females. Glomerulonephrosis was increased in males in the 200 and 2000 ppm dose groups and in females in the 20 and 200 ppm dose groups. The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occur in both sexes exposed to 2000 ppm and likely begins at 200 ppm. Two lipomatous tumours were observed in the kidneys of high dose group males, but these are not statistically significant and do not demonstrate a dose-response.

Clinical signs of neurotoxicity (tremors, seizures apathy, prostration, dyspnoea, and bristling coats) were observed in repeated dose oral and inhalation studies in rat. For inhalation exposure, neurotoxicity appears to be the critical endpoint. However, effects became apparent immediately after dosing (see section 10.11). Therefore, these effects observed in dose repeated studies are considered acute and are not considered to be relevant for STOT RE classification.

10.12.2 Comparison with the CLP criteria

The following CLP criteria are laid down for STOT RE:

Category 1 (H372):
Substances that have produced significant toxicity in humans or that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to produce significant toxicity in humans following repeated exposure.
Substances are classified in Category 1 for target organ toxicity (repeat exposure) on the basis of:
reliable and good quality evidence from human cases or epidemiological studies; or observations from appropriate studies in experimental animals in which significant and/or severe toxic effects, of relevance to human health, were produced at generally low exposure concentrations.

Category 2 (H373)
Substances that, on the basis of evidence from studies in experimental animals, can be presumed to have the potential to be Harmful to human health following repeated exposure.
Substances are classified in Category 2 for target organ toxicity (repeat exposure) on the basis of observations from appropriate studies in experimental animals in which significant toxic effects, of relevance to human health, were produced at generally moderate exposure concentrations.

Category 1:
No adverse effects relevant to STOT RE classification occurred in the available studies below the guidance value for category 1 (≤ 10 mg/kg bw/day). Therefore, classification in Category 1 is not warrented.

Category 2:
Based on the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver (increased weight, clinical chemistry parameters related to liver damage) and kidney (glomerulonephrosis, pigment deposition, increased absolute and relative weight) effects occur in rat in both sexes. In dog effects on the liver were also evident in both sexes. Most of the liver effects are considered adaptive, except the single-cell necrosis that occurred in one high dose female in the dog study. However, as this occurred in only one animal and the effect was described as minimal, this is considered insufficient to warrant classification for liver effects.
The kidney effects are considered to be treatment related and are seen as adverse. Kidney effects occurred in the 18-weeks oral rat study at a LOAEL of 37.5 mg/kg bw/day which is below the equivalent guidance value of ≤ 70 mg/kg bw/day for STOT RE cat. 2, calculated according to Haber's rule. Based on the 2-year rat study, a LOAEL of 9.99 mg/kg bw was derived which is below the equivalent guidance value of ≤ 125 mg/kg bw/day for STOT RE cat. 2. Based on the LOAEL values for kidney effects observed in rats and the equivalent guidance values, classification as STOT RE Cat. 2 is warranted.

10.12.3 Conclusion on classification and labelling for STOT RE

Classification as STOT RE Cat. 2 (H373: May cause damage to kidney through prolonged or repeated exposure).

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

Summary of the Dossier Submitter's proposal

To evaluate the specific target organ toxicity (repeated exposure) the dossier submitter listed twelve studies in rats, mice, dogs and rabbits. Based on these studies liver and kidney are the main targets for repeated dose toxicity.

In the 18-week rat study, centrilobular hepatic hypertrophy (minimal or moderate) appeared in most animals in the high dose group, 5000 ppm (equal to 384.1 and 515.4 mg/kg bw/day for males and females, respectively). Minimal centrilobular hypertrophy was also seen in the

500 ppm group (equal to 37.5 and 47.3 mg/kg bw/day for males and females, respectively). Liver enzyme levels were statistically significantly increased in both sexes in the 5000 ppm group with the exception of P450 enzymes in female rats. In the higher dose groups, liver weights were significantly increased (> 10%) and enlarged livers were observed in males only (3/10 and 2/10 at dose levels of 500 and 5000 ppm, respectively). These effects were considered to be adaptive and not adverse.

Absolute and relative kidney weights were increased in males in the 500 and 5000 ppm dose groups.

A 3-month dog study (Doc IIIA/Section A 6.4.1/02) also revealed centrilobular liver hypertrophy in all animals in the high dose group. Liver weights were increased (> 10% based on absolute weight), liver enzymes were induced and centrilobular hypertrophy was observed but no lipid vacuolation was reported. One female in the high dose group showed minimal single-cell necrosis in the liver.

In the 2-year rat study, seven of ten males in the 2000 ppm (equal to 100.4 and 142.1 mg/kg bw/day for males and females, respectively) dose group were found to have rough kidney surfaces. Absolute and relative kidney and liver weights were increased in males and females in the high dose groups. At the 12-month interim autopsy, absolute kidney weight in females in the 200 ppm group (equal to 9.9 and 13.6 mg/kg bw/day for males and females, respectively) was elevated. Glomerulonephrosis was seen in males in the 200 and 2000 ppm dose groups, yellow-brown pigment deposits were seen in the tubular epithelial cells and interstitial tissue of the kidneys of both male and female animals in the 200 and 2000 ppm dose groups in an apparent dose-dependent manner.

At the 24-month final autopsy, absolute kidney weight was increased in males and females in the 200 ppm dose group, relative kidney weight was increased in males in the 200 ppm group and relative liver weight in all treated females. Glomerulonephrosis was increased in males in the 200 and 2000 ppm dose groups and in females in the 20 and 200 ppm dose groups. The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occur in both sexes exposed to 2000 ppm and likely begins at 200 ppm.

Comments received during consultation

Two MSCAs commented on STOT RE. One MSCA supported classification as STOT RE 2; H373 (kidneys). The other MSCA asked to explain the justification regarding the adaptive nature of the liver effects in more detail. The DS responded that the observed liver effects below classification limits for STOT RE 2 were reversible effects that are induced by many substances. (CLP guidance: *"In some cases the adaptive response may also be associated with pathological changes which reflect the normal response of the target tissue to substances: for example, liver hypertrophy in response to enzyme induction")*. The DS noted that it would have been different if more severe histopathological effects would have been noted.

Hepatotoxicity of sufficient severity to fulfil the criteria for classification was observed in dogs and mice but not at dose levels below guidance values for classification.

Assessment and comparison with the classification criteria

The DS summarized twelve studies for the evaluation of STOT RE: five studies in rats (oral and inhalation), one in mice (oral), three in dogs (oral), one in rabbits (dermal), and two teratogenicity studies (rat / rabbit). The seven most relevant studies are compiled in the following table.

Table*: animal studies relevant for STOT RE classification (modified from table 21 from CLH report).*

Study, guideline	Dose levels, Test animals	Results	Equivalent guidance values	Reference
28-day oral rat study OECD TG 407	0, 10, 50, 250 mg/kg bw/day Wistar rat, 30/ sex/group (except high dose group which had 35/sex)	- Principal findings were transient appearance of tremor and seizures in 2 animals, and death of 7 animals in the high dose group - Liver enzyme induction in males - Increase in liver weight in males and females in the top dose group - Relative kidney weight in high dose group males (7.6%) and low/high dose group females (4.5%/9%) significantly increased NOAEL: 50 mg/kg bw/day LOAEL: 250 mg/kg bw/day, based on tremors, seizures, mortality and increased relative liver weight (17-20%)	≤ 30 mg/kg bw/day (Cat. 1) ≤ 300 mg/kg bw/day (Cat. 2)	Doc. IIIA/ Section A6.3.1
18-week oral rat study US EPA FIFRA	0, 10, 50, 500, 5000 ppm Males: 0, 0.8, 3.5, 37.5, 384.1 (397.2 in satellite group) mg/kg bw/day Females: 0, 0.9, 4.4, 47.3, 515.4 (487.5 in satellite group) mg/kg bw/day	Liver: - Increased relative liver weights in both sexes at 500 and 5000 ppm group (14% and 44% in males, 17% and 28% in females) - Enlarged livers in males at 500 and 5000 ppm (3/10 and 2/10 vs. 0/10 in controls) - Centrilobular hypertrophy in both sexes at 500 and 5000 ppm (8/10 and 10/10	≤ 7.0 mg/kg bw/day (Cat. 1) ≤ 70 mg/kg bw/day (Cat. 2)	Doc. IIIA/ Section A 6.4.1/01

	Wistar rat, 10/sex/group (except control and 5000 ppm group which had an additional 10/sex/group)	in males vs. 0/10 in controls, 4/10 and 9/10 in females vs. 0/9 in controls) Kidney, thyroid: - Relative kidney weight was increased in males at 500 and 5000 ppm (11% and 14%), - Thyroid hypertrophy in males at 500 and 5000 ppm (10/10 and 10/10 vs. 0/10 in controls) NOAEL: 50 ppm (3.5 mg/kg bw/day) LOAEL: 500 ppm (37.5 mg/kg bw/day) based on liver and kidney changes in both sexes		
3-month oral dog study OECD 409	0, 50, 350, 2500 ppm Equivalent to: 0, 1.9, 14, 93 mg/kg bw/day Beagle dog, 4/sex/group	- Increased liver weights in both sexes in the high dose group, liver enzymes were induced and centrilobular hypertrophy was observed, no lipid vacuolation - Lipids, cholesterol and triglyceride levels were increased - Increased thyroid weights and decreased levels of thyroid hormones in females NOAEL: 350 ppm (14 mg/kg bw/day) LOAEL: 2500 ppm (93 mg/kg bw/day) based on liver effects in both sexes	≤ 10 mg/kg bw/day (Cat. 1) ≤ 100 mg/kg bw/day (Cat. 2)	Doc IIIA/ Section A 6.4.1/02
13-week inhalation rat study OECD 413	Nominal dose: 0, 40, 250, 1000 mg/m^3 Analytical dose: 0, 4.9, 46.7, 220.2 mg/m^3 Wistar rat, 10/sex/group (except vehicle control and	- Post-exposure hyperactivity in all animals in the 1000 mg/m^3 group throughout the entire exposure period - In the first week animals in the highest dose group also showed ruffled and ungroomed fur and tremor after exposure - Combined results of the haematology,	≤ 20 mg/m^3 (Cat. 1) ≤ 200 mg/m^3 (Cat. 2)	Doc IIIA/ Section A 6.4.3

	1000 mg/m^3 which had additional 10 animals/sex/group "satellite groups") Duration of exposure: 6 h	clinical chemistry and urinalysis evidenced minor effects which might indicate slight liver and kidney effects, but these results are not supported by the histopathological findings NOAEL: 46.7 mg/m^3 (17 mg/kg bw/day) LOAEL: 220.2 mg/m^3 (79 mg/kg bw/day)		
2-year oral rat study OECD 453	0, 20, 200, 2000 ppm Males: 0, 1.0, 9.9, 100.4 mg/kg bw/day Females: 0, 1.4, 13.6, 142.1 mg/kg bw/day Wistar rat, 70/sex/group	- Rough kidney surfaces were noted in high dose group males - Glomerulonephrosis and pigment deposits within the kidneys were increased in the 200 and 2000 ppm dose groups (incidences for controls, low, mid and high dose groups: - males: 76%, 78%, 90%, 97% and females: 19%, 30%, 35%, 22% of rats , respectively) - Urinary bladder urothelial hyperplasia, thyroid follicular hyperplasia and increased cuboidal cells (males and females) and urinary bladder tumours (papilloma and carcinoma), observed at 2000 ppm NOAEL: 20 ppm (1.0 mg/kg bw/day) LOAEL: 200 ppm (9.9 mg/kg bw/day) based on effects on kidney	≤ 1.2 mg/kg bw/day (Cat. 1) ≤ 12 mg/kg bw/day (Cat. 2)	Doc IIIA/ Section A 6.5/01, Doc IIIA/ Section A 6.7/01
2-year oral mouse study OECD 451	0, 10, 100, 1000 ppm Males: 0, 2.1, 19.7, 199.5 mg/kg bw/day Females: 0, 3.1, 33.3, 279.0 mg/kg bw/d	Results from the haematological and clinical chemistry studies combined with histopathology suggest that liver damage occur in both sexes exposed to 1000 ppm and may begin at 100 ppm in	≤ 1.2 mg/kg bw/day (Cat. 1) ≤ 12 mg/kg bw/day (Cat. 2)	Doc IIIA/ Section A 6.5/02, Doc IIIA/ Section A 6.7/02

	B6C3F1 mice, 60/sex/group (+10 extra for rats/sex/group for 0 and 1000 ppm)	females, liver weights were increased and increased cholesterol levels were seen at 1000 ppm NOAEL: 10 ppm (2.1 mg/kg bw/day) LOAEL: 100 ppm (19.7 mg/kg bw/day for males, 33.3 mg/kg bw/day for females), based on liver damage in both sexes		
1 year oral dog study OECD 452	0, 30, 300, 3000 ppm, equivalent to 0, 1, 10, 100 mg/kg bw/d Beagle dog, 4/sex/group	In the higher dose groups relative and absolute liver weights were increased, no histopathological changes were seen in the liver or any other organ in treated animals In top dose animals N-demethylase levels were elevated and bilirubin levels were decreased, these effects support indication of liver effects NOAEL: 300 ppm (10 mg/kg bw/day) LOAEL: 3000 ppm (100 mg/kg bw/day)	≤ 2.5 mg/kg bw/day (Cat. 1) ≤ 25 mg/kg bw/day (Cat. 2)	Doc IIIA/ Section A 6.5/ 03

Liver

The 18-week oral rat study showed that treatment with transfluthrin affects liver, kidneys and thyroid. Relative liver weight was increased in both sexes (males: 14% and 44%, females: 17% and 28%) at 500 ppm and 5000 ppm, respectively. Dietary concentrations of 500 ppm are equivalent to 37.5 mg/kg bw/day (male) and 47.3 mg/kg bw/day (female) and concentrations of 5000 ppm are equivalent to 384 mg/kg bw/day (male) and 515 mg/kg bw/day (female).

In addition, in the 3-month oral dog study, increased liver weights, enzyme induction and centrilobular hypertrophy were recorded in the high dose group (2500 ppm, 93 mg/kg bw/day).

The 2-year mouse study reported increased liver weights and increased cholesterol levels at 1000 ppm [equivalent to 199.5 mg/kg bw/day (males) and 279.0 mg/kg bw/day (females)] as well as increased incidence of liver nodes in females and increased hypertrophy of periacinal hepatocytes at this dose.

These effects are above the guidance value for classification in Category 2. No effects were reported at doses below guidance value.

Liver effects can be regarded as adaptive responses (CLP Guidance, p. 474). Such compensatory changes can be associated with pathological changes. Liver hypertrophy is a normal response to enzyme induction. According to the guidance document, such adaptive responses are not considered toxicologically relevant and do not support classification.

Kidney

In the 2-year oral rat study effects on kidney were reported. Absolute kidney weight was increased in the mid and high dose group males and in the mid dose group females. A decreased urine density (at six months) and increased water consumption were observed in males of the 200 and 2000 ppm dose groups. Rough kidney surfaces were noted in 7/10 males in the 2000 ppm dose group and glomerulonephrosis was observed at an increased incidence in the 200 and 2000 ppm dose groups males compared to controls. A dose-dependent pigment deposition in the tubular epithelial cells and interstitial tissue was seen in the kidneys of male and female rats in the 200 and 2000 ppm dose groups.

Table*: glomerulonephrosis observed in the two-year study in rats*

	0 ppm		**20 ppm**		**200 ppm**		**2000 ppm**	
Non-neoplastic changes in kidney	m	f	m	f	m	f	m	f
Glomerulonephrosis	45/59	11/59	47/60	18/60	53/59	21/60	56/58	13/60
Pigment deposition	41/59	33/59	41/60	40/60	53/59	54/60	58/58	59/60

Conclusion

RAC agrees with the dossier submitter that the main target organs for repeated dose toxicity are liver and kidney.

Based on the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction; it is suggested that liver (increased weight, clinical chemistry parameters related to liver damage) and kidney (glomerulonephrosis, pigment deposition, increased absolute and relative weight) effects occur in rats in both sexes. In dogs, effects on the liver were also evident in both sexes.

RAC agrees with the DS that the observed liver effects can be regarded as an adaptive response. Thus, they do not support a classification.

RAC considers the renal effects observed not sufficiently robust for classification. In the 2-year oral rat study, glomerulonephrosis and pigment deposition also occur in the control groups at high incidences. These effects are not statistically significant in comparison to the control. Changes in kidney weights were also observed in the 28-day oral rat study, where kidney weight in males and females of the high dose group was found to be transiently increased and urine was found to contain epithelial cells. The authors of the 18-week oral rat study also reported increased kidney weight in males at 500 and 5000 ppm.

According to the CLP Regulation, increased organ weight is not a criterion for classification as STOT RE: "Annex I: 3.9.2.8.1. It is recognized that effects may be seen in humans and/ or animals that do not justify classification. Such effects include […] (c) Changes in organ weights with no evidence of organ dysfunction." Since no severe organ dysfunction could be detected, RAC is of the opinion that kidney effects do not warrant classification.

The kidney effects were limited to increased kidney weights, augmented glomerulonephrosis and pigment deposition. Glomerulonephrosis and pigment deposition occurred without statistically significant differences. Overall, RAC is of the opinion that classification as STOT RE 2; H373 (kidney) as proposed by the DS is not warranted.

10.13 Aspiration hazard

Not evaluated in this dossier.

11 EVALUATION OF ENVIRONMENTAL HAZARDS

The environmental hazards of transfluthrin were assessed in the Assessment Report (March 2014), addenda and Proposed Decision of the Netherlands prepared in the context of the approval under Regulation (EU) No 407/2014. Studies considered valid in the CAR (reliability score of 1 or 2) have been included in this report and were considered for classification purposes. The full study summaries as published in the CAR are available in Annex I. All studies were carried out under GLP unless indicated otherwise. The non-GLP studies were range-finding studies or mechanistic studies. Other than the mechanistic studies all studies reported in this section were carried out in accordance with OECD guidelines. Minor deviations were noted in some cases but these did not affect the overall reliability of the studies. The deviations are included in the summaries were relevant.

11.1 Rapid degradability of organic substances

Table 22: Summary of relevant information on rapid degradability

Method	Results	Remarks	Reference
Ready biodegradability OECD TG 301F; non-GLP; purity not reported; non-radiolabelled transfluthrin	Not readily biodegradable. 0% after 28 days (based on oxygen consumption)	Limited information provided on materials & methods and results sections. Test concentration of 100 mg/L exceeded water solubility. Reliable with restrictions (= Klimisch score of 2).	Kanne (1990); Document IIIA/ Section A7.1.1.2.1
Hydrolysis EPA Pesticide Assessment Guidelines, Subdivision N: § 161-1 (1982)	pH 5, 25 °C: hydrolytically stable pH 7, 25 °C: hydrolytically stable pH 9, 25 °C: DT50 14 days	Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity is acceptable. No repetition of the hydrolysis was conducted. Reliable with restrictions (= Klimisch score of 2).	Hellpointer, E. (1989).; Document IIIA/ Section A7.1.1.1.1
Water/sediment degradation study Similar to OECD TG 308 ; ^{14}C-transfluthrin; radiochemical purity 98.7->99%.	$DT_{50,water:}$ < 7 days (20°C) $DT_{50,system}$: 11.1 days (17.7 and 10.5 d; 20 °C) $DT_{50,sediment}$: 14.1 days (14.8 and 7.3 d; 20°C) Metabolites:	Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity is acceptable. No information on LOD/LOQ is given. Number of time points (total	Hellpointner, E. (1993); Document IIIA/ Section A7.1.2.2.2

Method	Results	Remarks	Reference
	NAK-4455 (TFB-OH): max. 37.8% in water after 7 days) $DT_{50,\ system}$: < 14 days NAK-4723 (TFB-COOH): (max.59% in water phase and. 81.2% in whole system after 70 days). NAK-4723 appears very persistent, even though reliable DT_{50} could not be derived..	5) is small, too small to estimate half-life for major metabolite NAK 4723 (TFB-COOH). No information on LOD/LOQ is given. Number of time points (total 5) is small, too small to estimate half-life for major metabolite NAK 4723 (TFB-COOH). Reliable with restrictions (= Klimisch score of 2).	
Phototransformation in water OECD Guideline 316	No reliable information on aqueous or soil photolysis is available, but direct photolytic degradation in water is not expected to be a relevant route of degradation of transfluthrin in water.	-	Hellpointner; Document. IIIA/ Section A7.1.1.1.2/03
Phototransformation in air (estimation method), including identification of breakdown products Guideline EC Directive 94/37/EC Guideline EC Directive 95/36/EC	Half-life: 2.429 days (24-hr day; 0.5E6 OH/cm^3) Half-life: 1.620 days (12-hr day; 1.5E6 OH/cm^3)]	Reliable (= Klimisch score of 1). Model calculation without deficiencies	Hellpointer, E. (2005); Document. IIIA/ Section A7.3.1
Biodegradation in soil OECD Guideline 317	Parent-DT50: 5.17 d (12°C), Metabolite NAK 4723 (2,3,5,6-tetrafluorobenzoic acid, BCS-AA52185)-DT50: 3.23 d (12°C), Formation fraction: 0.6190	Reliable (= Klimisch score of 1).	Reinken et al. (2015). Submitted for product authorisation and not included in CAR.

11.1.1 Ready biodegradability

A manometric respiratory test on ready biodegradability of transfluthrin (purity not reported) was performed according to OECD TG 301F (Kanne, 1990). Test concentration was 100 mg/L, which exceeds water solubility. Oxygen consumption in the blank vessels reached 49 mg/L after 28 days, satisfying the validity criterion of OECD 301F (≤60 mg/L). Degradation of reference material (aniline) calculated from oxygen consumption was 78% within 14 days exposure, which exceeds the required 60%. After 28 days, oxygen consumption in the transfluthrin vessels reached 39 mg/L, corresponding to 0% degradation. As this is below that observed in the blank vessels, it could indicate transfluthrin toxicity towards the inoculum. In the AR it was concluded that microbial toxicity is not likely, as in an activated sludge respiration test (Müller, 2001; Doc IIIA7.4.1.4) no inhibitory effect of transfluthrin was observed at concentrations up to 10 000 mg/L. The AR noted that a toxicity control (transfluthrin+reference substance) was lacking, and that the lower oxygen consumption was observed in only 1 of the duplicates. A scientific explanation for the observed effect at 100 mg/L could thus not be determined, and no definitive conclusion could be drawn with as to whether the classification *not readily biodegradable* is correct or is due to the test conditions. Considering all above, the AR concluded that repetition of the experiment is not considered necessary and that classification as not readily biodegradable can be accepted as worst-case. The

Dossier Submitter agrees with this conclusion. The results are considered reliable with restrictions, and are assigned a Klimisch score of 2, and are used for classification purposes.

11.1.2 BOD_5/COD

No data available.

11.1.3 Hydrolysis

Transfluthrin is hydrolytically stable at 25 °C, pH 5 and 7. The $DT_{50,hydrolysis}$ at pH 9, 25 °C is 14 days (Hellpointer, 1989). There is no reliable information on aqueous or soil photolysis. Furthermore, the notifier submitted a waiver for not repeating an aqueous photolysis study on transfluthrin concluding that direct photolytic degradation in water is not expected to be a relevant route of degradation of transfluthrin in water.

11.1.4 Other convincing scientific evidence

No data available.

11.1.4.1 Field investigations and monitoring data (if relevant for C&L)

No data available.

11.1.4.2 Inherent and enhanced ready biodegradability tests

No data available.

11.1.4.3 Water, water-sediment and soil degradation data (including simulation studies)

Biodegradation in water/sediment system

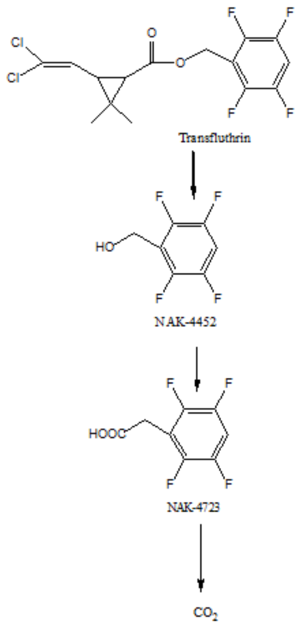
An aerobic water-sediment simulation study similar to OECD TG 308 is available (Hellpointner 1993). No significant deviations from the guidelines were reported. Test duration was 100 days. Two replicates in the dark and one replicate in the light were sampled at day 1, 7 ,28 ,70 and 100. Stock solution of 190 mg/L transfluthrin in ethanol was prepared. Mineralisation after 100 days was 3.0 and 12.6 % of AR for the respective systems. In two natural water/sediments systems, the dissipation of transfluthrin from the water phase was dominated by sorption, the dissipation $DT_{50,water}$ was reported to be < 7 days. The average degradation $DT_{50,system}$ was 11.1 days, the $DT_{50,sediment}$ 14.1 days.

Metabolites NAK 4452 (2,3,5,6-tetrafluorobenzyl alcohol; TFB-OH) and NAK 4723 (2,3,5,6-tetrafluorobenzoic acid; TFB-COOH) were detected in amounts > 10 % of AR in the water phase, maximum levels were 38 and 59% of AR, respectively. The same metabolites were found in sediment, maximum level was 2.9% of AR for TFB-OH and 26% of AR for TFB-COOH. Bound residues after 100 days were 4.4 and 7.9% of AR, mineralisation after 100 days was 3.0 and 12.6% of AR for the respective systems.

The DT50,system of metabolite TFB-OH was estimated to be < 14 days, a reliable estimate of the DT50, system of metabolite TFB-COOH could not be obtained because of few data points. Analytical results obtained in the water/sediment system indicate that metabolite TFB-COOH has a low degradation rate and is persistent in a water/sediment system. A DT50,system could not be derived. Notifier proposes 485 and 437 days, however, a value of 1000 days is proposed by evaluator.

The test was conducted in accordance with OECD TG 308, no significant deviations from the guidelines were reported. Reliability index = 2.

Figure 7.1.2.2.2-01: Degradation pathway of ^{14}C-transfluthirn in aerobic aquatic sediment systems



Biodegradation in soil

A simulation study for soil was performed according to OECD guideline 307, The degradation route and rate of transfluthrin was studied in four different German soils (abbreviated as AX, DD, HH and WW) under aerobic conditions in the dark at 20±2°C. The test substance was radiolabelled (purity not reported). Test duration was 14 days. Test concentration was 45 µg/kg soil dry weight. The test was performed in static systems consisting of Erlenmeyer flasks each containing 50 g soil (dry weight equivalents) and equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds. Duplicate samples were processed and analysed 0, 0.25, 1, 2, 3, 7 and 14 days after treatment (DAT). Total recovery for the four soils ranged 82.2 to 103.4% applied radioactivity (% AR). The maximum amount of carbon dioxide was 68.5, 78.3, 72.5 and 72.9% AR at study end (DAT-14) in soil AX, DD, HH and WW, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.2% AR at all sampling intervals for all soils. The losses of radioactivity observed throughout the study course were further investigated in additional tests and it ewas concluded that the insufficient material balances were caused by losses of carbon dioxide during sample processing. Non-extractable residues (NER) increased from DAT-0 to DAT-7 from 1.1 to 8.9% AR in soil AX, from 2.3 to 12.1% AR in soil DD, from 1.5 to 10.8% AR in soil HH and from 1.2 to 10.0% AR in soil WW. From DAT-7 to DAT-14 NER slightly decreased to 7.6% AR in soil AX, 10.4% AR in soil DD, 8.9% AR in soil HH and 8.6% AR in soil WW. One degradation product, i.e. NAK 4723[1] (2,3,5,6 tetrafluorobenzoic acid, BCS-AA52185), was identified in all investigated soils and with a maximum occurrence of 36.5% AR at DAT-2 in soil HH. For transluthrin (parent) the DT50 normalized to 12°C was 5.17 days, and for the transformation product NAK 4723 (2,3,5,6-tetrafluorobenzoic acid, BCS-AA52185) the DT50 normalized to 12°C was 3.23 days, see Table 23 below.

[1] Also called TFB-COOH in the consultation

Table 23: Modelling endpoints of transfluthrin and NAK 4723 as well as formation fractions

	Model	**χ2**	**$DT_{50-modelling}$ (20°C)**	**$DT_{50-modelling}$ (12°C)**	**f.f.**
Laacher Hof					
Parent	FOMC	3.98	1.93	3.66	
NAK 4723	SFO	13.8	1.93	3.66	0.6231
Whole model		7.68			
Dollendorf II					
Parent	FOMC	4.38	1.29	2.45	
NAK 4723	SFO	10.5	1.79	3.40	0.6130
Whole model		7.17			
Höfchen					
Parent	DFOP	0.87	18.3	34.71	
NAK 4723	SFO	10.1	1.6	3.03	0.7512
Whole model		6.03			
Wurmwiese					
Parent	FOMC	6.46	1.28	2.4	
NAK 4723	SFO	16.1	1.53	2.9	0.4886
Whole model		10.0			
DT50 (geometric mean)			Parent: 2.76 NAK 4723: 1.71	Parent: **5.17** NAK 4723: **3.23**	
f. f. (arithmetic mean)					**0.6190**

Field dissipation

No data available.

11.1.4.4 Photochemical degradation

No reliable information on aqueous or soil photolysis is available. Transfluthrin shows not any UV-absorption in the environmentally relevant wavelengths occurring on earth's surface. Therefore it can be regarded as stable with respect to direct phototransformation in water. Thus direct photolytic degradation in water is not expected to be a relevant route of degradation of transfluthrin in water.

The atmospheric half-life time of transfluthrin of 2.4 days is estimated according to TGD (2003) and based on 24-hr day and 0.5E6 OH/cm³ (Hellpointer, 2005).

11.2 Environmental transformation of metals or inorganic metals compounds

Not relevant for this dossier.

11.2.1 Summary of data/information on environmental transformation

Not relevant for this dossier.

11.3 Environmental fate and other relevant information

11.3.1 Adsorption/Desorption

A batch equilibrium study on the sorption behaviour of transfluthrin in soil is not available. Adsorption was estimated using the HPLC-method according to OECD TG 121. The adsorption coefficient was estimated using the relationship between retention times and log K_{OC}-values for a number of reference compounds A log K_{oc} of 4.7 was determined at pH 6. These data indicate the tendency of transfluthrin to bind to organic matter in soil and sediment.

11.3.2 Votalisation

Transfluthrin has a vapour pressure of 9 x 10^{-4} Pa at 20°C, indicating relatively low volatility. Its water solubility is low (0.057 mg/L), giving a calculated Henry's law constant of 5.86 Pa/m^3/mole. These data indicate that the substance has a tendency to volatilise from water.

EPI Suite version 4.0 reports a Henry's law constant of 2.6 Pa/m^3/mole at 20°C based on an experimental database structure match, and a HENRYWIN (v3.20) predicted Henry's law constant of 2.48 Pa/m^3/mole at 25°C. These values are slightly lower than reported in the AR, but are in the same range. EPI suite further provides an idea as to the volatility of transfluthrin from natural waters, i.e. a volatilization half-lives of 46 and 662 hours are predicted for a river (1 meter deep and a current velocity of 1 meter/second and a wind velocity of 5 meters/second), and a lake (1 meter deep with a current velocity of 0.05 meters/second and a wind velocity of 0.5 meters/second), respectively.

11.3.3 Distribution modelling

The CAR contains no information on environmental distribution of transfluthrin and the main environmental compartment receiving transfluthrin. Environmental distribution can be estimated using the fugacity model in EPI Suite v4.0, which is a Level III multimedia fate model that uses environmental parameters identical to those used in MacKay *et al*. 1992. The model is reduced to four main compartments, namely, air, water, soil and sediment. The distribution of the chemical and the environmental compartments depends on how the chemical is introduced. The table below provides an overview of environmental distribution assuming different emission scenarios. Input parameters were based on estimations within EPI Suite except for vapour pressure and water solubility which were taken from the AR. These fugacity modelling suggests that binding to soil/sediment is a more relevant process than evaporation from water.

Table 24. Level III fugacity modelling for transfluthrin

Release *	**Predicted environmental distribution**			
	Air	**Water**	**Soil**	**Sediment**
Equal emission to air, water and soil	0.183%	3.25%	72.3%	24.2%
100% emission to air	2.19%	1.02%	89.2%	7.60%
100% emission to water	0.0757%	11.5%	3.08%	85.4%
100% emission to soil	0.00041%	0.0159%	99.9%	0.118%
Equal emission to air and water	0.522%	9.27%	21.2%	69.0%
Equal emission to air and soil	0.224%	0.118%	98.8%	0.88%
Equal emission to water and soil	0.0229%	3.43%	71.0%	25.5%

*All calculations were based on a release of 1000 kg/hour to each compartment.

11.4 Bioaccumulation

Table 25: Summary of relevant information on bioaccumulation

Method	Results	Remarks	Reference
Bioconcentration in aquatic organisms (Bluegill Sunfish (*Lepomis macrochirus*)) Radiolabel: [Methylene-14C] Radiochemical purity: > 99% (HPLC, TLC) Chemical purity: > 99% (HPLC, UV) OECD 305 (1996)	BCF: 1704 and 1861 L/kg $_{ww}$ in whole fish based on mean 6.95% lipid content (based on Total Radioactive Residue) Normalised to 5% lipid content: 1226 and 1339 L/kg	Klimisch score of 2	Document IIIA/ Section A7.4.2

11.4.1 Estimated bioaccumulation

No data available.

11.4.2 Measured partition coefficient and bioaccumulation test data

A value of 5.46 was determined for the log Pow using the shake flask method, but this method is only valid for log Pow values between -2 and 4 (occasionally up to 5). Estimated values using BioLoom (BioByte, 2006) and Epiwin v3.2, are 5.94 and 6.17, respectively (Study A6.9/04). A new study was provided in the context of the CLH procedure after substance approval following the evaluation according to the requirements of Directive 98/8/EC. This study estimated using the HPLC method as described in OECD guideline 117 (pH 4, 7 and 9 at 25°C) for transfluthrin a log Pow of 5.5 . The latter value will be used for classification purposes.

A bioaccumulation study is available in accordance with OECD 305 (1996) (Document IIIA/ Section A7.4.2). The study was performed in 2 parts; Part 1 was a 42-day phase to examine the bioconcentration and depuration of [methylene-^{14}C]-Transfluthrin by bluegill sunfish *(Lepomis macrochirus)*, and Part 2 was a 7 to 14-day exposure to investigate the biotransformation of [methylene-^{14}C]-Transfluthrin in fish.

At exposure concentration 198 ng/L (bioconcentration study), concentrations of transfluthrin in the water phase decreased with time from 100 % of the total radioactive residue (TRR) at t=0 to 90.6 % at t=2 and 66.6 % at t=28. Concentrations of TFB-OH increased concurrently from n,d, at t=0 to 9.4 % of TRR at t=2 and 33.4 % at t=28.

At 132 ng/L (biotransformation study), transfluthrin accounted for 79.2 % of TRR at t=7 and 84.3 % at t=14, corresponding values for TFB-OH were 20.8 and 15.7 % of TRR.

No explanation is given for the fact that in the biotransformation study at 132 ng/L concentrations of transfluthrin are stable, this in contrast to the bioconcentration experiment at 198 ng/L.

BCF-values are estimated on the basis of TRR, because parent and metabolite concentrations in water and fish were not stable during the BCF-study. Based on TRR a steady state was reached. However, in the biotransformation part, concentrations of transfluthrin in water and fish were relatively stable. From the transfluthrin concentrations, a BCF of 1938 L/kg is calculated. In the CAR it was concluded that this figure is in good agreement with the BCF's based on TRR (1704 and 1861 L/kg at 65 and 198 ng/L). It was further stated that although the BCF of 1938 L/kg is less reliable because it is based on two time points only, the calculation do indicate that the BCF based on TRR can be used as a reliable estimate of the BCF for transfluthrin. All the BCF values reported in the CAR were based on a lipid content of 6.95%. The Dossier Submitter normalised the BCF values to a lipid content of 5 %, which is recommended in REACH Guidance R7c as it allows comparison between studies and comparison to the bioaccumulation criteria. The normalised BCF-values based on TRR are 1226 and 1339 L/kg at 65 and 198 ng/L, respectively.

The depuration time (DT90) is 10.1-12.7 days. The level of metabolites (%) in organisms accounting for > 10% of residues is for TFB-OH and TFB-COOH < 5%.

The fish showed no mortalities or abnormal behaviour throughout the study in all test vessels.

Data are considered reliable with restrictions, are assigned Klimisch score of 2 because of the instability of the test substance, and can be used for classification purposes.

Since BCF in fish ≥ 500 L/kg, transfluthrin can be considered as having a potential to bioconcentrate for classification purposes.

11.5 Acute aquatic hazard

Table 26: Summary of relevant information on acute aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Acute toxicity to fish flow through OECD TG 203	Rainbow trout (*Oncorhynchus mykiss*), old name (*Salmo gairdneri*)	NAK 4455 technical (transfluthrin) Purity: 94.5%	96 h LC_{50}: 0.7 µg/L	nominal (actual concentrations > 80 % of nominal) Klimisch score of 1 **Key data**	Document IIIA/ Section A7.4.1.1/01
Acute toxicity to fish Flow through OECD. 203	Golde orfe *(Leuciscus idus melanotus)*	NAK 4455 technical (transfluthrin) Purity: 94.5%	LC_{50}: 1.25 µg as/L	nominal (actual concentrations > 80 %) Klimisch score 1	Document IIIA/ Section A7.4.1.1/02
Acute toxicity to invertebrates static	*Daphnia magna*	NAK 4455 (transfluthrin technical)	EC_{50}: 1.7 µg/L	nominal Klimisch score 2	Heimbach, F. (1987); Document IIIA/

OECD 202		Purity: 95.0%			Section A7.4.1.2/01
Acute toxicity to invertebrates static OECD 202	*Daphnia magna*	NAK 4455 (transfluthrin technical) Purity: 95.7%	EC_{50}: 1.2 µg/L	mean measured Klimisch score of 1 **Key data**	Bruns, Dr. (2001); Document IIIA/ Section A7.4.1.2/02
Growth inhibition test on algae static OECD TG 201; limit test	*Scenedesmus subspicatus*	NAK 4455 (transfluthrin technical) Purity: 95.0%	ErC_{50}: > 57 µg/L NOErC ≥ 57 µg/L	Limit test; nominal, no effect observed at highest test conc.. Therefore, $E_rC50 > S_w$. Klimisch score of 1	Heimbach, F. (1987) ; Document IIIA/ Section A7.4.1.3/01
Growth inhibition test on algae static OECD 201	*Scenedesmus subspicatus*	NAK 4455 (transfluthrin technical) Purity: 95.7%	ErC_{50}: > 24.6 µg/L NOErC ≥ 9.6 µg/L	geometric mean measured Klimisch score of 1 **Key data**	Bruns, Dr. (2001); Document IIIA/ Section A7.4.1.3/02

11.5.1 Acute (short-term) toxicity to fish

The acute toxicity of transfluthrin to Rainbow trout (*Oncorhynchus mykiss*) was tested in a flow-through test according to OECD TG 203 (Document IIIA/Section A7.4.1.1/01). Nominal test concentrations were 0.16, 0.28, 0.50, 0.89, 1.58 and 2.81 µg a.i./l, plus control and solvent control (acetone 0.1 ml/1).. Fish were observed twice on the first day of exposure and daily thereafter (at 24, 28, 72 and 96 hours) for mortalities and signs of intoxication. Dissolved oxygen and pH were determined daily, temperature was measured hourly. Water hardness was determined at the beginning and at the end of the test. Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours in the concentrations 0.16, 0.28, 0.50 and 0.89 µg a.i./1. The concentrations 1.58 and 2.81 µg a.i./l were analysed at 0 and 24 hours.Water flow and dosing system were controlled twice daily and water flow was adjusted if necessary. Analytical results showed that test concentrations were maintained at >80% of the nominal values. In the highest test concentration, the mean value over 24 h was greater 80% of the nominal concentration with slightly lower values at start of the test. Hence, results refer to nominal values. Mortalities in the control, solvent control, 0.16, 0.28 and 0.50 µg a.i./l concentrations were 0%, respectively. 90% mortality was observed at 0.89 µg a.i./l and 100% mortality was observed at 1.58 and 2.81 µg a.i./l. Symptoms of intoxication such as swimming on side and/or inverted and staggering were noted in fish at dose levels of 0.89 and 2.81 µg a.i./l. Water quality and environmental parameters were within acceptable limits. The 96-hour LC50 of the test substance was calculated to be 0.7 µg a.i./l with a 95%-confidence interval from 0.62 to 0.79 µg a.i./l..

In addition, the acute toxicity of transfluthrin to Golden Orfe (*Leuciscus idus melanotus*) was determined in a flow-through test (Document IIIA/ Section A7.4.1.1/02). The concentrations tested were: 0.50, 0.89, 1.58, 2.81 and 5.00 µg a.i./l (nominal) plus control and solvent control (acetone 0.1 ml/1). Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours in the concentrations 0.50, 0.89 and 2.81µg/1. The concentrations 1.58 and 5.00 µg/1 were only analysed at 0 and 24 hours. Analytical results showed that test concentrations of 0.89, 1.58 and 2.81 µg a.i./l were maintained at a mean level of >80% of the nominal values. In the highest concentration (5.00 µg a.i./l) the initial measured concentration was slightly below 80 % of the nominal value. In the lowest concentration only 58-74% of the nominal concentration was recovered by analysis. It is considered that this had no influence on the study validity as at the next highest concentration 0.89 µg a.i./l no effects were observed. Hence, results of the study are reported as nominal concentrations. The 96-hour LC50 based upon nominal concentrations of the test substance was calculated to be 1.25 µg a.i./l with a 95 %-confidence interval from 1.1 to 1.4 µg a.i./l..

Data from both studies are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes.

11.5.2 Acute (short-term) toxicity to aquatic invertebrates

Two acute toxicity studies performed with *Dapnia magna* are available. In the first study, juvenile Daphnia magna (6 -24 hours old) were exposed for 48 hours under static test conditions to transfluthrin technical at nominal concentrations of 0.0010, 0.0018, 0.0032, 0.0056 and 0.010 mg a.i./L (Heimbach, 1987). Daphnids were observed for immobilisation and sublethal effects at 24 and 48 hours. Dissolved oxygen, temperature and pH were measured at the start and end of the study. Mortalities in the control and solvent control were 0 and 3%, respectively. After 24 h, 3%, 7% and 10 % of the daphnids were immobile at the test concentration of 0.0018, 0.0032, 0.0056 mg a.i./L. No effects were observed at 0.01 mg a.i./L at this time point. 7, 70 and 90% immobilisation was observed at the 0.0010, 0.00018 and 0.0032 mg a.i/l test concentrations after 48 h; 97% mortality was observed at the 0.0056 and 0.010 mg a.i./l test concentrations respectively. Water quality and environmental parameters were within acceptable limits. Based on this study, the 48-hour EC_{50} of transfluthrin was calculated to be 0.0017 mg a.i./l with 95%-confidence intervals of 0.0003 to 0.003 mg a.i./l. As no analytical measurements were conducted, the data are considered reliable with restrictions, and are assigned a Klimisch score 2.

In the second *Daphnia magna* study, Juvenile Daphnia magna (<24 hours old) were exposed for 48 hours under static test conditions to NAK 4455 (transfluthrin technical) at nominal concentrations of 0.0002, 0.0004, 0.0008, 0.002, 0.004, 0.008, 0.02 and 0.04 mg a.i./L (Bruns, 2001). An untreated control was also included in the study. Daphnids were observed after 24 and 48 hours for alteration of mobility and loss of locomotory actions. Dissolved oxygen, temperature and pH were measured at the end of the study. Water hardness was determined at the beginning of the test. Analytical samples were taken from the controls and the 0.002, 0.004, 0.008, 0.02 and 0.04 mg a.i./L test concentrations at 0 and 48 hours and analysed using GC. The test concentrations 0.0002, 0.0004 and 0.0008 mg a.i./L were not analytically determined as they were below the quantitation limit of the GC analysis method (0.001 mg/L). However at these test concentrations GC values were calculated (corresponding to the analytical recovery rate of the highest test concentration). Based on this study, the 48-hour EC50 of thransflutrin was calculated to be 0.0012 mg a.i./l with 95%-confidence intervals of 0.0008 to 0.0016 mg a.i./L. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes.

11.5.3 Acute (short-term) toxicity to algae or other aquatic plants

Two algal growth inhibition studies performed with the green algae *Scenedesmus subspicatus* are available. In the first study, *Scenedesmus subspicatus* was exposed to transfluthrin for a period of 96 hours; under static conditions (at 23 ± 1°C and 8000 lux constant illumination) at a nominal concentration of 0.1 mg a.i./L (Heimbach, 1987; Document IIIA/ Section A7.4.1.3/01). Acetone was used as solvent control. After 24, 48, 72 and 96 hours cell counts were photometrically (at a wave length of 578 nm) determined in individual test vessels. In addition modifications of the cell structure were monitored; additional cell samples were taken at random from one flask at each of the treatment levels and the controls at each time-point and examined microscopically for abnormalities. Temperature and pH were monitored daily. No inhibition of cell biomass or growth rate was observed at the test concentration 0.1 mg a.i./l compared to the controls. No abnormalities such as alterations of the cell structure were observed. Water quality and environmental parameters were within acceptable limits. The validity criteria were met in the control. In fact, the growth rate factor after 3 days was found to be >16 in 72h. Moreover, according to the study report the composition of the 10 times concentrated nutrient solution was found to be in line with required values and the EC-values of the reference standard $K_2Cr_20_7$ for the biomass growth and the growth rate of the algae agree well with the results of a ring trial. The highest concentration did exceed water solubility of 0.057 mg/L. In the AR, the 72-hours ErC50 based on growth rate was determined to be > 0.1 mg/L. As no toxic effects were observed for biomass and growth rate even at the highest tested concentration of 0.1 mg a.i./L, Dossier Submitter can agree to express the E_rC50 as above water solubility, i.e. 72h-E_rC of > 0.057 mg/L, and the 72-hours NOE_rC as ≥ 0.057 mg/L. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes.

In the second study, *Scenedesmus subspicatus* was exposed to transflutrin for a period of 72 hours; under static conditions (at 23 ± 2°C and 6000 -10000 lux constant illumination) at nominal concentrations of 0.003, 0.006, 0.013, 0.025, 0.05 and 0.1 mg a.i./L (Bruns, 2001; Document IIIA/ Section A7.4.1.3/02). Control without test substance was included. Also a control vessel with substance (0.1 mg a.i./L) without algae was used to determine loss of substance due to algal uptake. 3 Replicates were performed per concentration and 6 replicates per control. After 24, 48 and 72 hours cell densities were measured in a microcell counter or alternatively by means of a microscopic counting chamber. Temperature and pH were measured at the start and end of the study. Analytical samples were taken from the controls and each test concentration at 0 and 72 hours and analysed using GC. Actual concentrations at t = 0 and t = 72 hours were 50 – 80 and 4.0 – 17 %, respectively. In the medium without algae, recovery was 81 and 75% of nominal after 0 and 72 hours, indicating that measurement in the other test concentrations were highly influenced by the presence of the algae. This is not unexpected in view of the strong sorptive characteristics of transfluthrin. The average measured concentration in the medium without algae was close to 80 %, therefore in the CAR it was considered justified to evaluate the effects on the basis of nominal concentrations. The 72-hours ErC50 based on growth rate was determined to be > 0.044 mg/L, and the 72-hours NOE_rC was 0.017 mg/L. The Dossier Submitter considers that binding to algae has resulted in lower exposure concentrations, and therefore the effect concentrations should be expressed as geometric mean values, i.e. 72-hours ErC50 of >24.6 µg/L and NOE_rC of 9.6 µg/L. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes.

11.5.4 Acute (short-term) toxicity to other aquatic organisms

No data available.

11.6 Long-term aquatic hazard

Table 27: Summary of relevant information on chronic aquatic toxicity

Method	Species	Test material	Results	Remarks	Reference
Chronic, fish OCSPP Guideline 850.1400, OECD Guideline 210 (2013).	*Pimephales promelas*	Transfluthrin Technical; Purity: 97.7%	NOEC: 0.399 µg/L	mean measured Klimisch score of 1	IUCLID IIIA 9.1.6.
OCSPP Guideline 850.1300, OECD	*Daphnia magna*	Transfluthrin Technical; Purity:	NOEC: 0.0175 µg/L	mean measured	IUCLID IIIA

Guideline 211 (2012).		97.7%	based on the number of neonates per adult	Klimisch score of 1	9.1.6.2
Growth inhibition test on algae OECD TG 201 (1984).	*Scenedesmus subspicatus*	NAK 4455 (transfluthrin technical) Purity: 95.0%	NOErC ≥ 557 µg/L	nominal; limit test Klimisch score of 2	Heimbach, F. (1987); Document IIIA/ Section A7.4.1.3/01
Growth inhibition test on algae OECD TG 201 ( )	*Scenedesmus subspicatus*	NAK 4455 (transfluthrin technical) Purity: 95.7%	NOErC ≥ 9.6 µg/L	geometric mean measured Klimisch score of 1	Bruns, Dr. (2001); Document IIIA/ Section A7.4.1.3/02

11.6.1 Chronic toxicity to fish

A chronic toxicity study in fish is available in which Fathead minnow (*Pimephales promelas*) eggs starting at <24 hours old were observed for hatch rate; young fish were assessed for abnormal behavior, physical changes, mortality and growth (length, weight) (IUCLID IIIA 9.1.6). The study duration was 36 days under flow through conditions.. The 36-day chronic toxicity of Transfkuthrin to early life stage of Fathead minnow (Pimephales promelas) has been studied under flow throw conditions. Fertilized eggs <24 hours old were exposed to blank, vehicle blank, and to test material with nominal concentrations (mean measured) of: 62.5 (28.0), 125 (53.0), 250 (95.0), 500 (190), and 1000 (399) ng a.i./L. The system was maintained at a temperature range of 23.8 to 25.8 and a pH of 7.2 to 8.1 . The hatching phase started at Day 0 and ended at Day 4. The 36-day exposure to Transfluthrin Technical resulted in an overall NOEC of 399 ng a.i./L. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes

11.6.2 Chronic toxicity to aquatic invertebrates

In a 21-day chronic test first instars of *Daphnia magna* (<24 hours old) were exposed to nominal (mean measured) concentrations of control (<1.0), solvent control (<1.0), 7.00 (4.02), 14.0 (8.85), 28.0 (17.5), 56.0 (35.7) and 112 (68.6) ng a.i./L for 21 days under flow-through conditions. Two replicates were alternately sampled at each interval (weekly). Approximately 1000 ml of test water were sampled for analysis. Samples were not stored before analysis. The NOEC and LOEC were calculated based on mean measured concentrations. The 21-day exposure to Transfluthrin technical resulted in a NOEC of 17.5 ng a.i./L based on the number of neonates per adult reproduction day. The lowest EC10 and associated 95% confidence limits was calculated to be 18.3 (12.8 to 55.9) ng a.i./L for the endpoint of adult dry weight. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes

11.6.3 Chronic toxicity to algae or other aquatic plants

Two growth inhibition tests with *Scenedesmus subspicatus* were performed in accordance to OECD 201 (Heimbach, 1987 and Bruns, 2001). For a summary of the studies please refer to section 11.5.3 presented above. Based on these studies the 72-hours NOE_rC was determined as ≥ 0.057 mg/L. Data are considered reliable, are assigned Klimisch score of 1, and can be used for classification purposes. In the second study, the 72-hours NOE_rC was found to be 9.6 µg/L when expressed as geometric mean value.

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11.6.4 Chronic toxicity to other aquatic organisms

No data available.

11.7 Comparison with the CLP criteria

11.7.1 Acute aquatic hazard

The criteria for Category Acute 1 in line with Table 4.1.0 (a) from the Guidance on the Application of the CLP Criteria are:

96 hr LC50 (for fish)	≤ 1 mg/l and/or
48 hr EC50 (for crustacea)	≤ 1 mg/l and/or
72 or 96 hr ErC50 (for algae or other aquatic plants)	≤ 1 mg/l.

Transfluthrin is a poorly water-soluble substance, 0.057 mg/L at 20°C. Acute toxicity data is available for all three taxa. In the available studies performed with fish, the lowest LC_{50} value was found to be 0.7 µg/L and is thus lower than 1 mg/L. The toxicity to crustacea and algae was also below 1 mg/L, amounting to 1.2 and >24.6 µg/L, respectively. Based on the lowest LC_{50} of 0.7 µg/L for mortality observed in *Onchorhynchus mykiss*, transfluthrin should be classified as Aquatic Acute 1; H 400, with an M-factor of 1000.

11.7.2 Long-term aquatic hazard (including bioaccumulation potential and degradation)

Transflutrhin is not readily biodegradable based on a guideline study performed according to OECD 301F. Although this study has some deficiencies (limited information was provided on materials & methods and results sections and the test concentration of 100 mg/L exceeded water solubility) it is classified as 'reliable with restrictions' and it can be used to conclude that for classification purposes the classification is not rapidly degradable. Transfluthrin is rapidly mineralised in soil. However as mineralisation is less significant in water and sediment, the substance cannot be considered rapidly degradable.

In addition, a HPLC study is available that reported a log Kow of 5.5. This value is reliable and indicates that the log K_{ow} will exceed the threshold of log K_{ow} >4.0). The experimentally determined BCF was found to be 1226 and 1339 L/kg ww after normalisation to 5% lipid content. As this exceeds the teshold of BCF ≥500 L/kg_{ww}, it can be concluded that for classification purposes transfluthrin has a high potential for bioaccumulation.

The criteria for Category Chronic 1 and 2 in the CLP Guidance for non-rapidly degradable substances for which adequate chronic toxicity data are available are:

<u>Category Chronic 1:</u>

- Chronic NOEC or ECx (for fish)	≤0.1 mg/l and/or
- Chronic NOEC or ECx (for crustacea)	≤0.1 mg/l and/or
- Chronic NOEC or ECx (for algae or other aquatic plants)	≤0.1 mg/l.

<u>Category Chronic 2:</u>

- Chronic NOEC or ECx (for fish)	> 0.1 to ≤ 1 mg/l and/or
- Chronic NOEC or ECx (for crustacea)	> 0.1 to ≤ 1 mg/l and/or
- Chronic NOEC or ECx (for algae or other aquatic plants)	> 0.1 to ≤ 1 mg/l.

Chronic toxicity data is available for all three taxa. For fish a NOEC of 3.99×10^{-4} mg/L was derived. The NOEC for crustacea was found to be 1.75×10^{-5} mg/L. For algae the lowest NOErC was determined to be ≥ 9.6×10^{-3} mg/L. Considering the lowest chronic value of $1.75 \times 10^{--5}$ mg/L, transflutrhin can be classified for chronic toxicity as Aquatic Chronic: H410 with an M-factor of 1000.

11.8 CONCLUSION ON CLASSIFICATION AND LABELLING FOR ENVIRONMENTAL HAZARDS

Acute (short-term) aquatic hazard: category Aquatic Acute 1, M-factor: 1000.

Long-term aquatic hazard: category Aquatic Chronic 1, M-factor: 1000.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The substance is currently listed in Annex VI of the CLP Regulation (EC) No 1272/2008 with a classification for environment hazard Aquatic Acute 1 (H400) and Aquatic Chronic 1 (H410). The Dossier Submitter (DS) proposed to add the M-factors of 1000 for both acute and chronic aquatic hazard classifications, based on a new interpretation/evaluation of existing data for aquatic toxicity.

Degradation

A hydrolysis study following EPA Pesticide Assessment Guidelines, Subdivision N: § 161-1 (1982) was run at pH 5, 7 and 9 and at 25°C. Transfluthrin was stable at pH 5 and 7, while at pH 9 hydrolysis was observed. A DT_{50} value of 14 days was determined.

No reliable information on aqueous photolysis is available but transfluthrin does not exhibit any UV-absorption in the environmentally relevant wavelengths occurring on earth's surface. Therefore, it can be regarded as stable with respect to direct phototransformation in water. Thus, direct photolytic degradation in water is not expected to be a relevant route of degradation of transfluthrin in water.

There is one non-GLP ready biodegradability test available for transfluthrin following OECD TG 301F (Manometric Respirometry) which resulted in 0% (based on oxygen consumption) degradation after 28 days. The test concentration was 100 mg/L, which exceeds water solubility.

An aerobic water-sediment simulation study was performed at 20°C (OECD TG 308, GLP). Mineralisation after 100 days was 3.0 and 12.6% AR for the respective systems. In two natural water/sediments systems, the dissipation of transfluthrin from the water phase was dominated by sorption, the dissipation $DT_{50,water}$ was reported to be < 7 days.

The average degradation $DT_{50,system}$ was 11.1 days, the $DT_{50,sediment}$ was 14.1 days. Metabolites NAK 4452 (2,3,5,6-tetrafluorobenzyl alcohol; TFB-OH) and NAK 4723 (2,3,5,6-tetrafluorobenzoic acid; TFB-COOH) were detected in amounts > 10% AR in the water phase, maximum levels were 38 and 59% of AR, respectively. The same metabolites were found in sediment, maximum level was 2.9% of AR for TFB-OH and 26% of AR for TFB-COOH. Bound residues after 100 days were 4.4 and 7.9% of AR, mineralisation after 100 days was 3.0 and 12.6% of AR for the respective systems. The $DT_{50,system}$ of metabolite TFB-OH was estimated to be < 14 days, a reliable estimate of the $DT_{50,system}$ of metabolite TFB-COOH could not be obtained because of insufficient data. Analytical results obtained in the water/sediment system indicate that metabolite TFB-COOH has a low degradation rate and is persistent in a water/sediment system. A $DT_{50,system}$ could not be derived.

Based on available data, the DS concluded that transfluthrin is considered as not rapidly degradable for classification purposes.

Bioaccumulation

The measured octanol-water partition coefficient (log K_{ow}) determined using shake flask method is 5.45, but this method is only valid for log K_{ow} values between -2 and 4 (occasionally up to 5). The log K_{OW} determined according to OECD TG 117 (HPLC method) is 5.5 at 25°C and it is not pH dependent. The log K_{OW} values estimated with BioLoom (BioByte, 2006) and Epiwin v3.2 are 5.94 and 6.17, respectively.

A bioaccumulation study in bluegill sunfish *(Lepomis macrochirus)* following OECD TG 305 showed BCF values of 1704 and 1861 L/kg w/w in whole fish, based on a mean lipid content of 6.95% (based on Total Radioactive Residue), while the BCFs normalised to 5% lipid content are 1226 L/kg and 1339 L/kg.

The DS concluded that for classification purposes transfluthrin has a high potential to bioaccumulate in aquatic organisms.

Aquatic Toxicity

Reliable aquatic toxicity data are available in the CLP Report, and a summary of the relevant information on aquatic toxicity is provided in the following table (the key endpoints used in hazard classification are highlighted in bold). Transfluthrin has been shown to be poorly water soluble (0.057 mg/L at 20°C).

Table*: summary of relevant information on aquatic toxicity of transfluthrin*

Method	**Species**	**Endpoint**	**Toxicity value (µg/L)**	**Reference**
Short-term toxicity				
OECD TG 203	*Oncorhynchus mykiss*	96 h LC_{50}	**0.7** nom	Document IIIA/ Section A7.4.1.1/01
OECD TG 203	*Leuciscus idus melanotus*	96 h LC_{50}	1.25 nom	Document IIIA/ Section A7.4.1.1/02
OECD TG 202	*Daphnia magna*	48 h EC_{50}	1.7 nom	Heimbach, 1987; Document IIIA/ Section A7.4.1.2/01

OECD TG 202	*Daphnia magna*	48 h EC_{50}	1.2 mm	Bruns, 2001; Document IIIA/ Section A7.4.1.2/02
OECD TG 201	*Scenedesmus subspicatus*	72 h E_rC_{50}	> 57 nom	Heimbach, 1987; Document IIIA/ Section A7.4.1.3/01
OECD TG 201	*Scenedesmus subspicatus*	72 h E_rC_{50}	> 24.6 mm	Bruns, 2001; Document IIIA/ Section A7.4.1.3/02
Long-term toxicity				
OCSPP Guideline 850.1400, OECD TG 210	*Pimephales promelas*	36 d NOEC	0.399 mm	IUCLID IIIA 9.1.6, BPD IIA/Annex VII.7.3, Annex 1 to CLH report section 4.4
OCSPP Guideline 850.1300, OECD TG 211	*Daphnia magna*	21 d NOEC (reproduction)	**0.0175** mm	IUCLID IIIA 9.1.6.2, BPD IIA/Annex VII.7.3, Annex 1 to CLH report section 4.4.4
OECD TG 201	*Scenedesmus subspicatus*	72 h NOE_rC	≥ 57 nom	Heimbach, 1987; Document IIIA/ Section A7.4.1.3/01
OECD TG 201	*Scenedesmus subspicatus*	72 h NOE_rC	≥ 9.6 mm	Bruns, 2001; Document IIIA/ Section A7.4.1.3/02

Note: nom – nominal concentrations; mm – mean measured concentrations.

<u>Acute toxicity</u>

Short-term aquatic toxicity data on transfluthrin are available for fish, invertebrates, and algae.

For fish, two studies with two different species (*Oncorhynchus mykiss* and *Leuciscus idus melanotus*) and performed according to OECD TG 203 were available in the CLH dossier. Rainbow trout (*Oncorhynchus mykiss*) was the most sensitive fish species tested, with a nominal 96 h LC_{50} value of 0.7 µg/L (test material concentrations were maintained at mean measured values > 80 %, so results are reported using nominal values).

Two acute toxicity studies performed with *Daphnia magna* and according to OECD TG 202 were provided for aquatic invertebrates. The lowest endpoint for invertebrates is mean measured 48 h EC_{50} of 1.2 µg/L.

Two acute toxicity studies were available for algae *Scenedesmus subspicatus*. Both studies were carried out according to OECD 201. The lowest endpoint for algae is geometric mean measured 72 h E_rC_{50} value of > 24.6 µg/L.

All the values are below the classification threshold value of 1 mg/L. Based on the lowest acute toxicity value of 0.7 µg/L for fish *Oncorhynchus mykiss*, the DS concluded that transfluthrin warranted classification as Aquatic Acute 1 with M factor of 1000.

<u>Chronic toxicity</u>

Long-term aquatic toxicity data on transfluthrin are available for fish, invertebrates, and algae.

For transfluthrin, there was only one study carried out according to OCSPP Guideline

850.1400 and OECD TG 210 available for fish, with a mean measured 36 d NOEC value of 0.399 µg/L for *Pimephales promelas*.

There was only one study carried out according to OCSPP Guideline 850.1300 and OECD TG 211 available for aquatic invertebrates, with a mean measured 21 d NOEC value of 0.0175 µg/L for *Daphnia magna*.

Two studies performed according to OECD TG 201 were available for algae *Scenedesmus subspicatus* in the CLH dossier. The lowest endpoint for algae is geometric mean measured 72 h NOE_rC value of > 9.6 µg/L.

The chronic aquatic classification proposed by the DS was based on water flea *Daphnia magna* toxicity study (21 d NOEC = 0.0175 µg/L) along with the understanding that the substance is not rapidly degradable and has a high potential for bioaccumulation. The DS concluded that transfluthrin warranted classification Aquatic Chronic 1 with an M-factor = 1000.

Comments received during consultation

One comment was received from an MSCA which agreed with the DS's proposal to classify transfluthrin as Aquatic Acute 1, M-factor=1000 and Aquatic Chronic 1, M-factor=1000.

Assessment and comparison with the classification criteria

Degradation

Transfluthrin is hydrolytically stable under acidic and neutral conditions, but unstable under alkaline conditions. The hydrolysis half-life for transfluthrin was 14 days at pH 9. Transfluthrin showed 0% degradation after 28 days in the ready biodegradation test following OECD TG 301F and is, thus, considered to be not readily biodegradable. The average degradation DT_{50} in two natural water/sediment systems under aerobic conditions at 20°C was 11.1 days. Low level of mineralization after 100 days was observed for transfluthrin and its metabolites in water/sediment systems. No information allowing classification assessment of the metabolites is available in the CLH report and therefore it cannot be demonstrated that the metabolites do not fulfil the criteria for classification as hazardous to the aquatic environment.

In conclusion, RAC considers the available information reliable and agrees with the DS that transfluthrin should be considered not rapidly degradable for the purpose of classification under CLP.

Bioaccumulation

RAC considers the available bioaccumulation information reliable and agrees with the DS that transfluthrin can be considered as bioaccumulative in aquatic organisms. The basis for this is that measured BCF values in fish are above the CLP criterion of 500 and this is supported by the log K_{ow} values, which are above the CLP threshold of 4.

Acute toxicity

RAC is of the opinion that reliable acute toxicity data are available for all three trophic levels (fish, daphnia, and algae). Fish are the most acutely sensitive group and the lowest result is a 96 h LC_{50} value of 0.0007 mg/L for rainbow trout *Oncorhynchus mykiss*. RAC notes that all $L(E)C_{50}$s for fish, invertebrates, and algae (see table above) are below the threshold value of 1 mg/L. Consequently, RAC agrees with the DS that transfluthrin warrants classification for acute aquatic hazards as Aquatic Acute 1; H400. As 0.0001 < $L(E)C_{50}$ ≤ 0.001 mg/L, an M-factor of 1000 is also warranted.

Chronic toxicity

Reliable long-term aquatic toxicity data are available for all three trophic levels. The lowest chronic effect value is derived from the crustacea *Daphnia magna* with a mean measured 21 d NOEC of 0.0000175 mg/L. As the value is below the threshold value of 0.1 mg/L and the substance is considered not rapidly degradable, RAC agrees with the DS that classification as Aquatic Chronic 1; H410 is warranted. As 0.00001 < NOEC ≤ 0.0001 mg/L, an M-factor of 1000 is also warranted.

In summary, based on the available reliable data, **RAC agrees with the DS that transfluthrin warrants classification as:**

Aquatic Acute 1; H400, M-factor = 1000

Aquatic Chronic 1; H410, M-factor = 1000

12 EVALUATION OF ADDITIONAL HAZARDS

Not evaluated in this dossier

13 ADDITIONAL LABELLING

According to the CLH criteria the following should be considered for EUH066:

EUH066 — Repeated exposure may cause skin dryness or cracking:

For substances and mixtures which may cause concern as a result of skin dryness, flaking or cracking but which do not meet the criteria for skin irritancy in section 3.2 of Annex I, based on either: — practical observations; or — relevant evidence concerning their predicted effects on the skin.

Based on the available repeated dose dermal rabbit study (Doc. IIIA/ Section A6.3.2, see section 3.12.1.2 in Annex I of the CLH report) several skin effects were observed including redness, scaling, encrustation, swelling, red patches, increased skin fold thickness, thickening of the epidermis, and hyperkeratosis. These effects are indications that a dry and cracked skin can occur following repeated exposure to transfluthrin. Transfluthrin does not meet the criteria for classification for skin irritancy and therefore classification with EUH066, repeated exposure may cause skin dryness or cracking, is proposed.

14 REFERENCES

All references included in the present CLH report refer to the studies presented in the CAR. A full reference list for all the studies from the CAR is presented in Document IIIA of the CAR. In addition, the following references were used in this CLH report.

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Anonymous (2010). The effects of treatment with transfluthrin and tetrafluorobenzoic acid on rat and human urothelial cell lines. University of Nebraska Medical Center, USA, Study No. 299, Document No. M-364266-01-1.

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LeBaron *et al.* (2014). Characterisation of nuclear receptor-mediated murine hepatocarcinogenesis of the herbicide pronamide and its human relevance. Toxicological sciences, 142(1), 2014, 74-92.

Okuda *et al.* (2017). Evaluation of the human relevance of the constitutive androstane receptor-mediated mode of action for rat hepatocellular tumor formation by the synthetic pyrethroid momfluorothrin. J. toxicol. Sci. vol. 42, no. 6, 773-788.

Reinken et al. (2015). Transfluthrin (BFT) Soil Kinetics for Modelling – Kinetic Evaluation of the Degradation of Transfluthrin and its Metabolite NAK4723 under Aerobic Laboratory Soil Conditions . Bayer CropScience AG, Monheim, Germany. Report No.: EnSa-15-0752. 2015-09-25/ M-534584-01-1

Yamada et al., 2009. Case Study: An Evaluation of the Human Relevance of the Synthetic Pyrethroid Metofluthrin-Induced Liver Tumors in Rats Based on Mode of Action. Toxicological sciences 108(1), 59–68

15 ANNEXES

The study summaries from the CAR of transfluthrin have been included in Annex I. In addition, study summaries of some studies identified in the CAR as ‘non-key studies’ are included in Annex I to provide a complete overview of the data available for transfluthrin.