Committee for Risk Assessment

RAC

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

Adopted

18 March 2021

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CLH-O-0000006955-61-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **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**

The proposal was submitted by **Netherlands** and received by RAC on **14 November 2019.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **9 December 2019**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **7 February 2020**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Ralf Stahlmann**

[Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **18 March 2021** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and 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 (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate	405-060-5	118712-89-3	Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H315 H400 H410	GHS07 GHS09 Wng	H315 H400			
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** Carc. 2 Acute Tox. 4 STOT SE 1 STOT RE 2 **Remove** Skin Irrit. 2	**Retain** H400 H410 **Add** H351 H302 H370 (nervous system) H373 (kidney) **Remove** H315	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H351 H302 H370 (nervous system) H373 (kidney) **Remove** H315	**Add** EUH066	**Add** oral: ATE = 583 mg/kg bw M=1000 M=1000	
RAC opinion	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** Carc. 2 Acute Tox. 4 STOT SE 1 **Remove** Skin Irrit. 2	**Retain** H400 H410 **Add** H351 H302 H370 (nervous system) **Remove** H315	**Retain** GHS07 GHS09 Wng **Add** GHS08	**Retain** H410 **Add** H351 H302 H370 (nervous system) **Remove** H315	**Add** EUH066	**Add** oral: ATE = 583 mg/kg bw M=1000 M=1000	
Resulting Annex VI entry if agreed by 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	Carc. 2 Acute Tox. 4 STOT SE 1 Aquatic Acute 1 Aquatic Chronic 1	H351 H302 H370 (nervous system) H400 H410	GHS07 GHS08 GHS09 Wng	H351 H302 H370 (nervous system) H410	EUH066	oral: ATE = 583 mg/kg bw M=1000 M=1000	

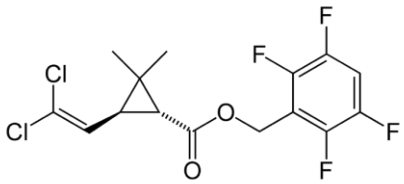
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Transfluthrin is intended for non-professional users as a fast-acting pyrethroid insecticide.

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.

HUMAN HEALTH HAZARD EVALUATION

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 $300 < ATE \leq 2000$ 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).

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

Method, guideline, deviations if any, species, strain, sex, no/group	**Route of exposure Relevant information about the study (as applicable)**	**Results**	**Reference**
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.

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.**

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,	Liver: - Increased relative liver weights in both sexes at 500 and 5000 ppm group (14% and 44% in males,	≤ 7.0 mg/kg bw/day (Cat. 1)	Doc. IIIA/ Section A 6.4.1/01

Study, guideline	Dose levels, Test animals	Results	Equivalent guidance values	Reference
	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 Wistar rat, 10/sex/group (except control and 5000 ppm group which had an additional 10/sex/group)	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 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	≤ 70 mg/kg bw/day (Cat. 2)	
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	Nominal dose: 0, 40, 250, 1000 mg/m^3 Analytical dose:	- Post-exposure hyperactivity in all animals in the 1000 mg/m^3 group throughout the entire exposure period	≤ 20 mg/m^3 (Cat. 1)	Doc IIIA/ Section A 6.4.3

Study, guideline	Dose levels, Test animals	Results	Equivalent guidance values	Reference
OECD 413	0, 4.9, 46.7, 220.2 mg/m^3 Wistar rat, 10/sex/group (except vehicle control and 1000 mg/m^3 which had additional 10 animals/sex/group "satellite groups") Duration of exposure: 6 h	- 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, 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)	≤ 200 mg/m^3 (Cat. 2)	
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 females,	≤ 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

Study, guideline	Dose levels, Test animals	Results	Equivalent guidance values	Reference
	B6C3F1 mice, 60/sex/group (+10 extra for rats/sex/group for 0 and 1000 ppm)	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 is not warranted.**

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* ($\leqslant$ 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)**.

ENVIRONMENTAL HAZARD EVALUATION

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.

Acute toxicity

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.

Chronic toxicity

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 $0.0001 < L(E)C_{50} \leq 0.001$ 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 $0.00001 < NOEC \leq 0.0001$ 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

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and RAC (excluding confidential information).

Annex 3 Records of the targeted public consultation following the submission of additional information (mechanistic studies, study summaries and expert statements) pertaining to the assessment of carcinogenicity mode of action of transfluthrin.