Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

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

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

CLH-O-0000001412-86-183/F

Adopted

5 December 2017

5 December 2017

CLH-O-0000001412-86-183/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: **cyflumetofen (ISO); 2-methoxyethyl (RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate**

EC Number: **-**

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

The proposal was submitted by the **Netherlands** and received by RAC on **14 December 2016.**

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

PROCESS FOR ADOPTION OF THE OPINION

The 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 **6 February 2017**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **23 March 2017**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Brendan Murray**

Co-Rapporteur, appointed by RAC: **Pietro Paris**

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 **5 December 2017** by **consensus**.

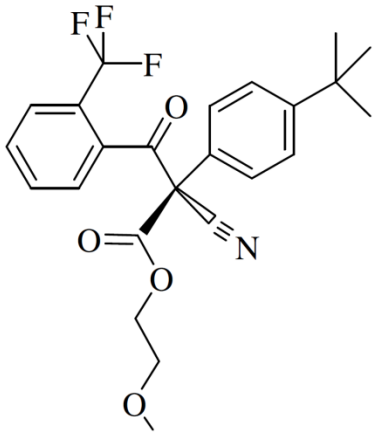
Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	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	No current Annex VI entry										
Dossier submitters proposal	TBD	cyflumetofen (ISO); 2-methoxyethyl (RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate	-	400882-07-7	Skin Sens. 1A Carc. 2	H317 H351	GHS07 GHS08 Wng	H317 H351			
RAC opinion	TBD	cyflumetofen (ISO); 2-methoxyethyl (RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate	-	400882-07-7	Skin Sens. 1A Carc. 2	H317 H351	GHS07 GHS08 Wng	H317 H351			
Resulting Annex VI entry if agreed by COM	TBD	cyflumetofen (ISO); 2-methoxyethyl (RS)-2-(4-tert-butylphenyl)-2-cyano-3-oxo-3-(α,α,α-trifluoro-o-tolyl)propionate	-	400882-07-7	Skin Sens. 1A Carc. 2	H317 H351	GHS07 GHS08 Wng	H317 H351			

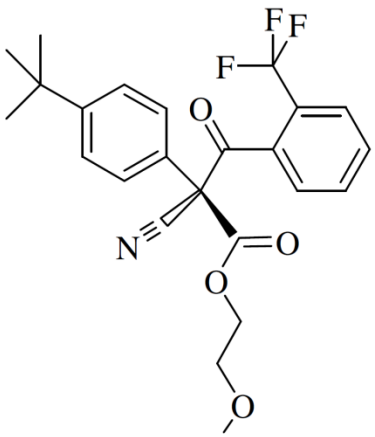
GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

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



S-cyflumetofen



R-cyflumetofen

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

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

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

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

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

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Acute Oral Toxicity

The oral LD_{50} was > 2000 mg/kg bw in female rats in a guideline compliant and GLP study. Male rats were not tested. According to CLP Regulation, LD_{50} values for acute oral toxicity > 2000 mg/kg bw do not warrant classification. The RAC is in agreement with the DS, cyflumetofen does not meet the criteria for classification and RAC therefore recommends **no classification for acute oral toxicity**.

Acute Inhalation Toxicity

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

Acute Dermal Toxicity

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

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

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

Classification as an eye irritant is required when a mean Draize score at or above 1 (corneal opacity or iritis) or 2 (conjunctival redness or conjunctival oedema) is observed from gradings at 24, 48 and 72 hours following installation of the test substance in at least 2 out of 3 animals.

No such effects for cyflumetofen were observed. RAC agrees with the DS that the substance **does not meet the criteria for classification for serious eye damage/irritation**.

RAC evaluation of respiratory sensitisation

Summary of the Dossier Submitter's proposal

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

As there are no data suggesting that cyflumetofen may cause respiratory sensitisation, RAC proposes **no classification for respiratory sensitisation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS summarised a single "well performed" GLP and OECD TG 406 compliant Guinea Pig Maximisation Test (GPMT) using 10 female Dunkin Hartley guinea pigs, with 5 female animals as controls (Hooiveld, 2003a). The concentrations of cyflumetofen used were 1% for intradermal induction, 50% for topical induction (preceded by pre-treatment with 10% SDS for 24 h) and 50% for the challenge. After topical challenge with 50% cyflumetofen, well-defined to moderate erythema was observed in 10/10 test animals after 24 and 48 hours, but no dermal reactions were seen in control animals. Since > 60% (100%) of treated animals responded to the treatment, and the intradermal induction concentration used in the study was 1%, the DS considered that cyflumetofen met the criteria for classification as Skin Sens. 1A. However, no SCL was proposed due to lack of evidence.

Comments received during public consultation

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

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

Assessment and comparison with the classification criteria

According to Annex I, Section 3.4.2.2.1.2 of CLP, where data are sufficient, a refined evaluation on the basis of the occurrence or potency of the sensitising effect can allow the allocation of skin

sensitisers into sub-category 1A (high frequency of occurrence, strong sensitisers), or sub-category 1B (a low to moderate frequency of occurrence, low to moderate potency).

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

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

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

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

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

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

Summary of the Dossier Submitter's proposal

In the CLH report, the DS summarised:

- One oral (dietary) 28 days and 13 weeks repeated dose toxicity (RDT) studies in CD-1 mice,
- six oral (dietary) RDT studies in Fischer F344 rats (ranging in duration from 14 days to 1 year),
- and three RTD studies in beagle dogs (oral, gelatine capsules; 28 days, 13 weeks and 12 months)

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

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

accumulation and deposition as a result of a reduction in hormone sensitive lipase and its ability to make cholesterol available from cholesteryl esters for steroid synthesis.

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

The vacuolation of interstitial ovary cells was not accompanied by any other histopathological changes or functional disturbance of the ovaries.

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

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

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

No RDT studies were conducted via the inhalation route.

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

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

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

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

Study	Criteria for STOT RE 2	NOAEL	LOAEL	Effects at LOAEL (Male/Female)	Reference
		mg/kg bw/d			
Oral studies					
Rat F344 14-day (dietary)	≤ 600	M: -- F: --	**101** **105**	↑ liver wt rel. (+10%*/--) ↑ adrenal wt abs. (--/+36%*) ↑ adrenal wt rel. (+25%*/+36%*) ↑ vacuolation of adrenal cortical cells (0/6 vs [6/6]/[6/6]) ↑ vacuolation of interstitial gland cells ovary (0/6 vs [--]/[6/6])	Sakai, 2001
Rat F344 26-day (dietary)	≤ 300	M: 43 F: 46	**128** **132**	↑ cortical cell vacuolation ([0/5] vs [3/5]/[3/5]) ↑ adrenal wt abs. (--/+34%**) ↑ adrenal wt rel. (--/+36%**)	Buesen, 2010
Rat F344 28-day (dietary)	≤ 300	M: 37.6 F: 40.8	**75.1** **79.8**	↑ adrenal wt rel (--/+15%*) ↓ triglycerides (-20%**/--) ↑ vacuolation of adrenal cortical cells (0/6 vs [6/6]/[6/6]) ↑ vacuolation of interstitial cells ovary (0/6 vs [--]/[2/6])	Yoshida, 2004a
Mouse CD-1 28-day (dietary)	≤ 300	M: 135 F: 150	663 763	↑ adrenal weight ↑ vacuolation and hypertrophy of adrenal cortical cells ↑ hyperplasia of adrenal subcapsular cells	Yoshida, 2004b
Dog 28-day (capsule)	≤ 300	M: 100 F: 100	**300** **300**	↑ vacuolation of adrenal cortical cells (0/3 vs [2/3]/[3/3]) ↑ adrenal wt abs (+16%/+28%) ↑ adrenal wt rel (+11%/+24%)	Nagashima, 2003a
Rat F344 90-day (dietary)	≤ 100	M: 16.5 F: 19.0	**54.5** **62.8**	↑ vacuolation of adrenal cortical cells (0/10 vs [6/10]/[--]) ↑ adrenal cortex hypertrophy (0/10 vs [--]/[10/10])	Yoshida, 2004c
Mouse CD-1 90-day (dietary)	≤ 100	M: 117 F: 150	348 447	↑ vacuolation of adrenal cortical cells ↑ adrenal cortex hypertrophy	Yoshida, 2004d
Dog 90-day (capsule)	≤ 100	M: 300 F: 300	1000 1000	↓ body weight gain ↑ adrenal and testis weight ↑ vacuolation adrenal cortex	Nagashima, 2003b
Dog 1-year (capsule)	≤ 25	M: -- F: --	30 30	↑ adrenal findings (mild vacuoles)	Nagashima, 2004 and 2008
Rat F344 1-year (dietary)	≤ 25	M: 18.8 F: 23.2	56.8 69.2	↑ vacuolation of adrenal cortical cells ↑ adrenal cortex hypertrophy ↑ vacuolation of interstitial cells ovary	Yoshida, 2004e
Rat F344 1-year (dietary)	≤ 25	M: -- F: --	250 319	↑ testis: interstitial cell hyperplasia (6/20 vs 19/20) ↑ M: vacuolation of adrenal cortical cells (1/20 vs 19/20) ↑ M: Pancreas: atrophy, acinar	Yoshida, 2012

				cell, focal (4/20 vs 14/20) ↑ vacuolation of interstitial cells ovary (0/20 vs 11/20)	
Dermal studies					
Rat Wistar 28-day	≤ 600	> 1000	--	No adverse effects	Buesen R. *et al.* 2010a

-- Not relevant (generally less than 10% of controls)
N.B. If there is no value for a particular effect it was because the data was not supplied in the CLH report or the LOAEL is outside of the upper bound trigger values for STOT-RE 2.
* $p \leq 0.05$; ** $p \leq 0.01$

Major Target Organs

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

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

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

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

Relevance of the effects for classification

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

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

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

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

The main target of cyflumetofen after repeated exposure is the adrenal gland (and steroidogenic tissues in general) as observed in rat, mouse and dog. The effect triggering the lowest endpoint was vacuolation of the adrenal cortical cells, which was observed in sub-acute, semi chronic and chronic toxicity studies. In the chronic studies the LOAEL for vacuolation of adrenal cortical cells was similar to the LOAEL observed in semi chronic studies, indicating no increase of the effect after prolonged exposure. When considering all the studies, significant or severe toxicity related to the vacuolation of the adrenal cortical cells was **not** a feature for any of the tested species. There was no indication of functional disturbance in all of the available toxicity data on cyflumetofen. Morphological changes could be profound at very high doses (e.g. mouse adrenals) but without apparent toxicity. There was no indication of significant impacts on animal health in any of the toxicity studies.

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

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

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

Comments received during public consultation

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

Assessment and comparison with the classification criteria

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

In Vitro *Studies*

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

An *in vitro* assay for gene mutations in L5178YITK mouse lymphoma cells (Verspeek-Rip, 2007), with and without S9 mix, was positive. Cyflumetofen concentrations from 1 to 333 µg/mL used in a range-finding test revealed high cytotoxicity at 100 µg/mL, with reduction of relative suspension growth by 97% and 31% in the absence and presence of S9 mix, respectively. At 333 µg/mL, little cell survival was observed. In the mutagenicity test, the following concentrations were chosen for the gene mutagenicity assay: 0, 20, 30, 40, 50, 60, 70, 80 and 90 µg/mL for treatment without S9 mix and 0, 10, 20, 40, 60, 80, 100, 120 and 140 µg/mL for treatment with S9 mix. Mutation frequency was approximately 2-, 2.7- and 3.3-fold increased relative to controls at 70, 80 and 90 µg/mL, respectively, in the experiment without S9 mix and at a mean of 2.5- and 6.2-fold relative to controls at 120 and 140 µg/mL, respectively, in the experiment with S9 mix. Moreover, cyflumetofen revealed an up to 4.5- and 1.6-fold increase (in the absence of S9 mix) and an up to 6.9- and 2.8-fold increase (in the presence of S9 mix) in the mutation frequency of small and large colonies, respectively, which may suggest that cyflumetofen could mainly induce chromosomal aberration; this was, however, not confirmed by the *in vitro* chromosomal aberration studies in hamster cells and the *in vivo* micronucleus assay in mice.

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

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

In Vivo *Studies*

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

Overall weight of evidence

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

Table: Summary of results from genotoxicity studies with cyflumetofen

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

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

Comparison with the criteria

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

There are no data from *in vivo* heritable germ cell mutagenicity tests showing mutagenic effects in germ cells of humans therefore a classification as Muta. 1B is precluded. Cyflumetofen is negative in acceptable *in vitro* tests with the exception of the mouse lymphoma thymidine

kinase (TK) gene mutation assay, and *in vivo* somatic cell mutagenicity guideline tests in mammals. However, the overall weight of evidence suggests no concern for mutagenicity.

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

Overall, RAC agrees with the DS that **classification is not warranted for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

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

- one 18 month study in CD-1 mice (Yoshida, 2004) supplemented with a GLP OECD TG 451 compliant single high dose level study (Yoshida, 2013)
- one 24 month study in Fischer F344 rats (Yoshida, 2004) supplemented with a GLP OECD TG 451 compliant single high dose level study (Takahashi, 2013).

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

Non-neoplastic changes in mice

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

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

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

Mortality, clinical signs, body weight, food consumption and haematology were not affected by treatment. A substance related increase in the incidence of diffuse vacuolation of adrenal cortical cells was noted in mice treated at ≥ 483 mg/kg bw/day (tables 84, 87, CLH report). There was no evidence of hypertrophy (in contrast with rats). This was statistically significant and outside the historical control range. At 1132 mg/kg bw/day, females showed significant increases in the incidences of pale-coloured skin and pale-coloured eye/eyelid. In organ weight, females showed statistically significant increases in absolute and relative weights of the

adrenals (table 86, CLH report, +39, +42% relative to controls respectively). The incidence of oligospermia was increased relative to controls at the highest dose only (15/52 vs 24/52).

Neoplastic changes in mice

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

Non-neoplastic changes in rats

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

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

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

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

Neoplastic changes in rats

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

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

There was also an increased incidence of C-cell carcinomas of the thyroid observed in male rats at the highest dose only (220 mg/kg bw/day). There was no increase in C-cell hyperplasia. There was a non-statistical increase in C-cell adenoma at the high dose relative to controls, and statistical significance was observed only for the combined incidence of C-cell

adenomas/carcinomas, and then only when all animals were included (i.e. animals scheduled for termination at week 104 as well as animals killed in extremis). The DS considered this a substance-related effect.

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

Comments received during public consultation

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

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

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

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

Assessment and comparison with the classification criteria

Technical equivalence of batches used in the long-term studies in rats

IET conducted two carcinogenicity studies in rats with cyflumetofen. One study was conducted at doses up to 62 mg/kg bw/day (Yoshida, 2004), while a second high dose study was conducted at 0 and 220/287 mg/kg bw/day (Takahashi, 2013). The batches of cyflumetofen were very similar, batch 01D1 from the 2004 study was 97.67% pure while batch 09/0510-3 (009025) from the newer technical material was 97.82% pure. A confidential report evaluated the batches used in 2012-2013 against the original specification of cyflumetofen in Vol. 4 of the DAR as agreed during the EU review of the substance (statement on the impurity profile of Batch 09/0510-3 (009025) used in the MTD studies in rats and mice, Pluijmen, 2014). The report concluded that the levels of impurities in the newer technical material were representative of those in the older batches on which the DAR specification was based.

Relevance of tumours observed in long-term studies in rats

Neoplastic findings included an increased incidence of interstitial cell benign adenoma of the testis (Leydig cell tumour, LCT) with statistical significance at the highest dose (96% vs 76% in control animals). This incidence was also outside the range of recent (5-year) IET HCD (56-76%; mean 68%). It is also noteworthy to point out that all tested doses of cyflumetofen resulted in incidences of LCT outside the range of the IET laboratory HCD without a discernible dose response.

An increased incidence of C-cell carcinoma of the thyroid gland occurred in males but not in females. The increased incidence of C-cell carcinoma in males (31% vs 18% in control animals) was not statistically significant, but clearly exceeded the IET laboratory historical control range (6-18%; mean 14%).

Leydig cell tumours

Table: Incidence of F344 rat interstitial cell tumours, numbers of animals affected

Dose in mg/kg bw/day	**0[1]**	**0[2]**	**4.9[1]**	**16.5[1]**	**49.5[1]**	**220[2]**
All animals examined	43/50	38/50	42/49	43/48	46/50	**48/50****

[1] Yoshida, 2004; [2] Takahashi, 2013; ** p ≤ 0.01 (Fisher's exact probability test)

Table: Incidence of F344 rat interstitial cell tumours in IET HCD

Year	**2008**	**2009**	**2011**	**2012**	**2013[1]**
Incidence (animals affected)	36	31	28	36	38
n	50	50	50	50	50

[1] Takahashi, 2013 control.
Mean: 34 ± 4; range 28 – 38; normally distributed.
Older studies (1993 – 2005) show incidences from 35 – 49 animals affected out of 50.

Table: Incidence of F344 rat interstitial cell hyperplasia in 1 year study, numbers of animals affected, 12 month time point

Dose in mg/kg bw/day	**0[1]**	**0[2]**	**1.9[1]**	**5.6[1]**	**18.8[1]**	**56.8[1]**	**250[2]**
Animals examined	0/20	6/20	0/20	0/20	0/20	0/20	**19/20****

[1]. Yoshida, 2004; [2]. Yoshida, 2012; ** p ≤ 0.01 (Fisher's exact probability test)

The Leydig cell tumour incidence was higher than concurrent and HCD. The mutagenicity tests do not support a genotoxic mode of action for cyflumetofen. Hyperplasia is the proliferative precursor lesion of LCT. LCT begin as Leydig cell hyperplasia (LCH), and the distinction between hyperplasia and adenoma is only based upon size (i.e. a mass of interstitial cells with a diameter greater than three seminiferous tubules is considered an adenoma). The only study where an increased incidence of LCH was observed was in the high dose (250 mg/kg bw/day) 1-year chronic rat study (table Incidence of F344 rat interstitial cell tumours, numbers of animals affected", above). This incidence is increased over the HCD (incidence of 12 month LCH within the last five relevant years at IET is 2/20 – 1/20 – 0/20 – 9/20) and concurrent controls (6/20). When considered along with the early onset of hyperplasia (at the high dose in the 1-year

chronic study, Yoshida, 2004), the increased incidence of LCT at the high dose in the 2y cancer study may be considered treatment related, but this is against a significantly high background. Also, it is noted that by the end of the 15 month study period nearly all F344 rats will have either hyperplasia or adenomas (Boorman *et al.,* 1990), so establishing a substance related effect under these conditions must be considered with caution.

Five *in vitro* mechanistic studies were performed to identify a possible mode of action for LCT via perturbation of the oestrogen or androgen system.

1. Aromatase assay: cyflumetofen was not an inhibitor of CYP19 aromatase activity.
2. Oestrogen receptor activation: an *in vitro* human ERα-transcriptional activation system showed cyflumetofen was negative in two out of three assays and only marginally positive (RPCMax: 10.4%) in the remaining assay. Considered negative overall.
3. *In vitro* ER-binding assay: cyflumetofen was considered to be "not interactive" for ER binding.
4. *In vitro* AR binding assay: Marginal displacements of [^{3}H]R1881 (a synthetic androgen, also called methyltrienolone), at concentrations at or near precipitation of cyflumetofen were observed, cyflumetofen was considered to be "not interactive" for AR binding.
5. *In vitro* H295R steroidogenesis assay: cyflumetofen demonstrated an apparent influence on the steroidogenesis pathway, acting as an inducer of estradiol production and an inhibitor of testosterone production in this test system. The biological significance of the small changes in steroid metabolism is considered equivocal, especially in the context of absent significant similar changes or adverse consequences *in vivo*. In addition, if cyflumetofen does act on the adrenal glands to affect hormone sensitive lipase activity then perturbations in other steroidogenic tissues such as testes and ovary are to be expected. The significance of the effects in this assay are therefore considered uncertain.

The mode of action remains unclear. Indeed, it is debateable if it is meaningful in the context of a lesion (LCT) that occurs in the F344 rat where that lesion's background incidence is so high that it is not usually considered relevant to other species, including humans, or even to other rat strains. Generally this is the case for LCT in the Fisher F344 male rat (section 3.6.2.2.6-a of the CLP Guidance). In 2004, the Specialized Experts Meeting of the European Chemicals Bureau agreed that "data on LCTs generated in Fisher rats or other rat strains having comparably high spontaneous LCT rate are considered normally not informative" (ECBI/08/04 Add 4, 2004).

Leydig cell tumours are extremely common in the F344 rat, with a typical background incidence of 75-100% in 2-year cancer studies. Humans are orders of magnitude less sensitive to the development of this tumour type. The F344 rat is particularly susceptible to this process and is not considered a relevant model for studying LCT. Because of the differences present between the human and rat Leydig cells, non-genotoxic compounds that cause LCT in rats generally have low relevance to humans. RAC therefore considers the LCT incidence for F344 rats dosed with cyflumetofen not to be relevant for classification purposes.

Thyroid C-cell tumours

Table: Incidence of male F344 rat Thyroid C-cell tumours (all animals)

Dose in mg/kg bw/day	**0[1]**	**0[2]**	**4.9[1]**	**16.5[1]**	**49.5[1]**	**220[2]**
Incidence:						
Adenoma, C-cell	9	11	8	4	11	15
Carcinoma, C-cell	6	9	7	2	4	15
A/C, C-cell	14	19	15	6	14	**28***
n	50	50	50 (23)	50 (15)	50	49

[1] Yoshida, 2004; [2] Takahashi, 2013

 (shaded cells) The incidences reported for doses 4.9 mg/kg bw/day and 16.5 mg/kg bw/day may be underestimates, only 23 and 15 animals examined respectively out of a total of 50 in each dose group, i.e. only animals with macroscopic thyroid lesions were accessed. However, these doses are so low that they may be expected to reflect the normal control incidence. This would appear to be the case.

* $p \leq 0.05$ (Fisher's exact probability test)

Table: Incidence of male F344 rat Thyroid C-cell tumours in IET HCD

Year	**2008**	**2009**	**2009**	**2011**	**2012**	**2013[1]**
Incidence:						
Adenoma, C-cell	21	22	10	14	18	11
Carcinoma, C-cell	7	3	5	9	7	9
A/C, C-cell	25	25	13	20	21	19
n	50	50	50	50	50	50

[1] Takahashi, 2013 control.
Adenoma Mean: 16 ± 5; range 10 – 22; normally distributed.
Carcinoma Mean: 7 ± 2; range 3 – 9; normally distributed.
A/C Mean: 21 ± 4; range 13 – 25; normally distributed.

Table: Incidence of male F344 rat Thyroid C-cell hyperplasia

Dose in mg/kg bw/day	**0[1]**	**0[2]**	**4.9[1]**	**16.5[1]**	**49.5[1]**	**220[2]**
Incidence	24	17	2	4	17	18
n	50	50	50 (23)	50 (15)	50	49

[1] Yoshida, 2004; [2] Takahashi, 2013

 (shaded cells) The incidences reported for doses 4.9 mg/kg bw/day and 16.5 mg/kg bw/day may be underestimates, only 23 and 15 animals examined respectively out of a total of 50 in each dose group.

The incidence of thyroid C-cell adenoma in high dose (220 mg/kg bw/day) male rats was greater than the concurrent control levels (15/49 high dose vs 11/50 for the control, Takahashi, 2013), and was not statistically significant ($p = 0.125$). C-cell adenomas occur frequently and with a large variation in Fisher F344 rats and this may reduce concern with respect to their

human relevance. The HCD clearly illustrates that the observed adenomas fall within the normal background (mean 32% or 16/50 animals, range 10 – 22/50) expected from this rat strain observed in the same testing facility as that used to generate the cyflumetofen data. In isolation, the adenoma incidence data do not provide reliable evidence of a treatment related carcinogenicity.

The incidence of thyroid C-cell carcinoma male rats exposed to high dose (220 mg/kg bw/day) was greater than the concurrent control levels (15/49 high dose vs 9/50 for the controls) and also greater than the HCD at the testing institute (mean 14% or 7/50 animals, range 3 – 9/50). A Fisher's exact test performed by RAC at all dose levels was significant ($p = 0.003$). The key question therefore becomes: is the incidence of C-cell carcinoma at the 220 mg/kg bw/day dose level sufficient to justify classification for carcinogenicity category 2?

The incidence of combined C-cell adenomas and carcinomas is greater than both the concurrent control levels and HCD, with statistical significance.

There is no mechanistic data available to explain the pathogenesis for the development of the C-cell tumours observed in the high dose group. The data indicate that there is a progression towards malignancy; that overall neoplastic potential is associated with the high dose cyflumetofen group only. Limited evidence is thus available in animals to suggest a carcinogenic effect but a causal relationship cannot be stated emphatically. The key information for consideration by RAC is:

1. There is a well conducted single study in one species with one sex affected.
2. Malignant neoplasms (30.6%) occur more frequently than in concurrent controls (18%) and also lie outside of the testing laboratory's historical control data.
3. Benign neoplasms also show an increased incidence in the high dose relative to concurrent controls but this increase is within the HCD of the testing laboratory.
4. The particular tumours of interest (C-cell carcinomas) exhibit a moderate background incidence of 6 – 18% (2008 – 2012).
5. The tumour type is relevant for human hazard assessment (where it is known as Medullary Thyroid Carcinoma, MTC).

RAC considers that the incidence of C-cell carcinoma in the high dose rat carcinogenicity study with cyflumetofen (Takahashi, 2013) to be potentially substance related and this issue is discussed further below.

Treatment related effect or of spontaneous origin: consideration of weight of evidence

Consideration of a substance related effect

There are several important questions to note here, chief amongst them is why is there a lack of other supporting evidence that fits in with a presumed model of carcinogenesis that would normally be expected for a substance mediated effect, i.e. dose dependent proliferative lesions of C-cells resulting in the progression of focal C-cell hyperplasia to adenoma and thereafter carcinoma with increasing age? It may be because cyflumetofen exerts a subtle but substance-specific mode of action, or it could be due to an unspecific stimulation of these tumours in this strain of rats that results in a high spontaneous rate of tumours, or it could be simply sporadic in nature. In humans, thyroid C-cell tumours give rise to MTC. C-cell hyperplasia may precede and accompany sporadic MTC but it is also frequently absent except in the case of hereditary MTC. This implies there is no guarantee that C-cell hyperplasia will always precede MTC in humans. In the case of subtle effects or less sensitive species, there is no guarantee that C-cell hyperplasia would be a notable feature of tumourigenesis. Methodological factors may also

play a part because single random sections through a small organ like the rat thyroid may not record small tumours such as C-cell adenomas (Hardistry, 1985). Serial sectioning of this organ has been shown to improve the detection of a series of rat tumours including thyroid C-cell adenomas (Thompson & Hunt, 1963).

According to CLP Annex I, 3.6.2.2.6, a number of important factors (among others) may be considered which may increase or decrease the level of concern for carcinogenicity and the classification category.

1. Tumour type and background incidence - **Relevant**:
   By default, carcinogenic effects in experimental animals are considered relevant to humans. There is no evidence to indicate that C-cell tumours in rodents are not relevant to humans. Indeed humans exhibit C-cell tumours in the form of medullary thyroid cancer (MTC).

   The background incidence of C-cell carcinoma is moderate in male F344 rats and fairly stable within recent (5-year) IET laboratory historical controls. The effect in the high dose group is both above concurrent controls and greater than the upper bound limit of the HCD. Unlike Leydig cell tumours in this strain of rat, C-cell carcinomas are considered relevant for human hazard assessment. The presence of an increased incidence in tumours supports Category 1B but the moderate background level may reduce the biological significance for this neoplasm in the F344 rat. According to the latest CLH guidance, "… appearance of only spontaneous tumours, especially if they appear only at high dose levels, may be sufficient to downgrade a classification from Category 1B to Category 2, or even no classification…" In this case expert judgement considers a case for Category 2 or no classification to be plausible.

2. Statistically significant effects in comparison to the concurrent controls? – **Yes**
   Observed for both carcinomas and for the combined incidence of adenomas and carcinomas. Limited evidence for a carcinogenic effect - Category 2.

3. Is there a dose-dependency of the effect? – **No**
   Effects are only seen at the highest dose tested (220 mg/kg bw/day). Limited evidence for a carcinogenic effect - Category 2.

4. Are pre-neoplastic effects observed? – **No**
   No effect on C-cell hyperplasia was seen in any treatment group compared to the controls. No increase in calcitonin or Ki-67 labelling after 14 and 53 weeks of treatment. Low concern for carcinogenicity. No support for classification.

5. Multi-site responses – **No**
   One type of tumour (C-cells) is observed at only one site (thyroid) in one species (rat). This may be sufficient to downgrade a classification from Category 1B to Category 2.

6. Progression of lesions to malignancy – **Relevant**
   While the increase in C-cell adenomas is not considered significant per se, a treatment related increase in C-cell carcinomas is evident in the Takahashi study (2013) and indicates a progression towards malignancy. This could support Category 1B. In contrast, the lack of precursor proliferative lesions of the C-cell in shorter duration studies, or in studies with lower doses of cyflumetofen supports a spontaneous aetiology. Category 2 or no classification are possible.

7. Reduced tumour latency – **No**
   The data from scheduled interim necropsy and repeated dose studies indicates there is no reduction in tumour latency. No support for classification.

8. Are responses seen in a single sex or both sexes – **One Sex**

Only males showed an increased incidence of tumours following treatment in a non sex-specific tissue. ADME studies indicate there is no sex specific differences in toxicokinetics. Females received a higher dose (287 mg/kg bw/day) but did not present with a convincing tumour response. Additional data would be required to provide sufficient evidence for animal carcinogenicity (1B), a decreased level of biological significance in this case supports Category 2 or no classification.

9. Are responses seen in a single species or multiple species – **One Species**
   Only male rats from the F344 strain showed an increase in C-cell tumours at the high dose level (220 mg/kg bw/day) over a lifetime study. There was no record of C-cell tumours or C-cell lesions of any kind in the mouse lifetime studies. The mice were dosed up to 5-fold greater (1143/1132 mg/kg bw/day) than the doses used in the rat lifetime studies. Responses observed in a susceptible strain against a moderate spontaneous background supports Category 2 or no classification.

10. Structural similarity to a substance(s) for which there is good evidence of carcinogenicity – **Not Relevant**
    No data.

11. Routes of exposure – **Relevant, Oral**

12. Comparison of ADME between test animals and humans – **No Data**.

13. Possibility of a confounding effect of excessive toxicity at test doses? – **No**
    No effect on mortality was observed in the Takahashi study *(*2013). Body weights were decreased in the males by up to 7%. Body weight gain relative to controls over the entire test period was reduced by about 10%. Adrenal and testes weights were substantially increased. The study was designed to exceed the MTD. Category 2 or no support for classification.

14. Mode of action and its relevance for humans – **Unknown**
    See section 4 below for a more detailed assessment. The mode of action is unknown, but for compounds that induce C-cell tumours there is typically a common pattern of development of pre-neoplastic and neoplastic lesions, i.e. the progression from increased hyperplasia to adenoma and finally carcinoma. In addition, precursor lesions (hyperplasia and adenomas) typically have a higher incidence than the final malignant tumours. These sequences of events were not observed for cyflumetofen. Several mechanisms could be eliminated based on deductive reasoning and a lack of secondary effects. No specific data is available to propose a mode/mechanism of action. It is unlikely to be vitamin D_3 mediated, no chronic hypercalcaemia, and there is no data to support cyflumetofen acting on or enhancing GLP-1R receptor activity. Category 2 or no support for classification.

15. Supporting evidence from genotoxicity data – **No**.
    Based on the available mutagenicity tests, the mechanism for the carcinogenic effect of cyflumetofen is probably non-genotoxic. No support for classification.

16. Sufficient evidence (Carc. 1B) for carcinogenicity in animals:

    a. Evidence from two or more independent studies in one species carried out at different times or in different laboratories or under different protocols – **No**.

    b. Evidence derived from a clear increased incidence of tumours in both sexes of a single species in a well-conducted study, ideally conducted under GLP – **No**.

Consideration of no substance related effect

The case argued against classification and a substance mediated effect has a central tenet; namely, the increased incidence in C-cell carcinomas (Takahashi, 2013). It is most likely not treatment related but spontaneous in nature. This was based on a number of observations inconsistent with the idea of a substance promoted carcinogenic effect on the thyroid C-cells:

- No increased incidence in proliferative precursor lesions (hyperplasia, adenomas),
- The absence of proliferative events was confirmed by retrospective staining of thyroid tissue with the proliferative marker Ki67. No increased proliferation was observed for animals treated 3 months or 1 year with 250 mg/kg bw/day,
- The incidence for C-cell adenoma shows large variations in F344 rats,
- There is no dose response in the incidence of C-cell carcinomas,
- There is no change in tumour latency,
- There is no difference in the unilateral vs bilateral occurrence of C-cell carcinoma between the high dose group and concurrent controls – almost all the C-cell carcinomas that were observed, occurred unilaterally in both groups (12 of 15 treated-male rats vs 7 of 9 males),
- Effects are observed in male rats but not in females in the absence of a relevant sex specific difference in internal dose or toxicokinetics as shown in ADME studies,
- C-cell carcinomas are not seen in mice treated for up to 18 months at 1143/1132 mg/kg bw/day which is approximately 5 times the dose used in the rat lifetime study.

Mode of action (MoA) of C-cell tumour formation

A comprehensive overview of known modes of action for C-cell tumour formation were presented by industry in its public comments and supplied in the form of position papers evaluating the relevance of the C-cell tumours. The MoA is not known for cyflumetofen and was not investigated in any depth. A comparison with inducers known to promote C-cell tumours in rodents (ionizing radiation, chronic hypercalcemia (due to e.g. excess Vitamin D; excess dietary calcium intake or absorption; stimulation of C-cells induced by compounds enhancing GLP-1 receptor activity or acting as direct agonists), indicates that the increase in proliferative lesions of the thyroid follows a standard pattern of progression from hyperplasia via adenoma to carcinoma.

Disruption of calcium homeostasis

The studies available for cyflumetofen indicated no altered plasma calcium-levels when treated for up to one year. There was no treatment related pathological change in any calcium homeostasis-related organ like bone tissue, kidney or parathyroid. Therefore, a hypercalcemia related background is not considered likely.

GLP-1 receptor agonism of C-cells

Involvement of the GLP-1 receptor cannot be directly ruled out since calcitonin levels were not measured. However, based on a lack of expected secondary effects (altered blood glucose levels or increased incidence of C-cell hyperplasia), therefore this mode of action cannot be considered relevant given the absence of the expected secondary effects.

C-cell hyperplasia as a precursor lesion

It is highly plausible that all possible mechanisms involve C-cell hyperplasia as a precursor lesion to the development of C-cell tumours. None of the studies with cyflumetofen gives any indication of increased incidences of C-cell hyperplasia at any treatment time point. There were no indications of thyroid involvement when cyflumetofen was tested in a comprehensive set of studies in the rat, ranging from 28 days up to 1 year of treatment except for the 2-year study with a high dose level of 220 mg/kg bw/day. A supplemental 1-year toxicity study (Yoshida, 2012), was conducted at 0 and 250/319 mg/kg bw/day in male and female rats, which were killed after 4, 13, and 52 weeks of treatment. There were no proliferative lesions in the thyroid glands of either sex after 4 and 13 weeks of treatment. When the thyroids were examined after 52 weeks of treatment, C-cell hyperplasia was observed in 2 control males, 2 control females and 3 treated-females, and C-cell adenoma was observed in one control male and one treated-female. Of note, no proliferative lesions in the thyroids were found in the treated males.

Gender-specific differences in kinetics or metabolism

An increased incidence in C-cell tumours was only observed in male rats. Based on the available kinetic data from the high dose ADME studies reported in the DAR, female rats are expected to be exposed to higher levels of cyflumetofen or its metabolites than males. Furthermore, little to no difference is seen with respect to tissue distribution or total radioactive label in the thyroid from both sexes. There were no gender-specific differences in kinetics or metabolism to explain why only one sex was effected.

In conclusion, the mode of action for the formation of C-cell tumours in male rats dosed with 220 mg/kg bw/day remains unknown. RAC is of the opinion that thyroid C-cell tumours in the F344 rat cannot be easily disregarded in the context of human hazard assessment.

Comparison with the Criteria

Classification as *Category 1B is not supported,* since there is no firm evidence from animal experiments to demonstrate a strong substance related effect. There was no effect in the mouse, and the only relevant tumours were thyroid C-cell carcinomas in one sex (males) from one study (Takahashi, 2013) with no dose response (high dose effect only) and no substance related increase in pre-neoplastic lesions.

The increase in thyroid C-cell carcinoma could be due to stimulation of the high spontaneous rate of these tumours in this strain of rats. However, this is not shown. In any case, an increase in the incidence of a spontaneously occurring tumour is still subject to consideration and may be sufficient to downgrade a classification from Category 1B to Category 2, and also be sufficient to consider no classification.

As it cannot be excluded that the observed thyroid tumours are relevant for humans, these should be taken into account for classification of cyflumetofen for carcinogenicity.

RAC Conclusion

The evidence of carcinogenicity is restricted to a single experiment and single sex, at one high dose level and there is no support for carcinogenicity from studies in other species. The mouse lifetime studies recorded no C-cell lesions at any dose or time point, even though the top dose tested was approximately 5-fold higher than that in the rat lifetime study.

RAC was informed that the normal pathogenesis of C-cell carcinoma is a progression from hyperplasia to adenoma to C-cell carcinoma and that the precursor lesions (hyperplasia and adenomas) typically have a higher incidence than the final malignant tumours. However, it is evident that this model is not supported by the findings of both the Yoshida (2004) and Takahashi (2013) from rat lifetime studies. There was no indication of precursor proliferative

lesions of the C-cell in shorter duration studies, or in studies with lower doses of cyflumetofen, i.e. no dose response and no time course for development of the pre-neoplastic lesions and malignant tumours.

Methodological factors may influence the identification of proliferative lesions and the ability to distinguish them from adenomas, particularly in a small gland such as the rat thyroid (Hardistry, 1985). Adenomas are typically described as regions of hyperplasia greater than five follicles in width. Sectioning of the thyroid is critical for the identification of small tumours such as C-cell adenomas. Industry confirmed the standard method employed was a single random section through the gland. Serial sectioning of this gland has been shown to be a better technique in establishing the true incidence of small tumours and regions of hyperplasia (Thompson & Hunt, 1963). It is therefore possible that such information has been missed in these studies because serial sectioning of the thyroid was not employed.

Although the evidence for a treatment related effect is weak and there is no dose response relationship, there is however, a real signal above background. It is statistically significant at the highest dose tested in a susceptible rat strain.

There is therefore sufficient uncertainty for RAC to conclude that **classification as Carc. 2 (H351: Suspected of causing cancer) is warranted**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

In the CLH report, the DS summarised three oral, GLP compliant, reproductive toxicity studies; an OECD TG 416 two generation reproductive toxicity study in rats and two OECD TG 414 teratogenicity studies, one in rats and the other in NZW rabbits.

According to the DS, in the two generation study, the only effects that may be related to reproduction were a slight (but statistically significant and dose-dependent) delay in sexual maturation in males and females (which was within the historical control range for the rat strain used), statistically significant prolongation of the oestrous cycle length (but within the historical control range), an increase in ovary interstitial vacuolation (consistent with the RDT studies) and effects on the serum levels of FSH, progesterone and 17β-estradiol in females which were statistically significant but were of reduced statistical power due to the small sample size based on low animal numbers (no HCD were available). The DS considered that these findings were of doubtful toxicological significance and therefore proposed no classification for effects on fertility.

In the developmental toxicity studies, incomplete ossification was seen at a dose level of 250 mg/kg bw/day in rats and delayed ossification was seen at a dose level of 1000 mg/kg bw/day in rabbits. In both species some evidence of maternal toxicity was observed at these doses; increased adrenal weight and increased incidence of vacuolation of adrenal cortical cells in rats and decreased food consumption and body weight gain in rabbits. In neither species were irreversible structural effects reported. The DS proposed no classification for developmental effects.

Comments received during public consultation

A comment was received from one MSCA:

They proposed that cyflumetofen should be discussed with regard to classification for reproductive toxicity and that Category 2 for fertility (H361f) should be considered. This was based on their observation that, while no effects on reproductive and fertility index are observed

in the 2-gen study, delay in sexual maturation (possibly related to changes in steroidogenesis) is observed in both sexes in the absence of systemic toxicity. Note that there was no effect on bodyweight in all tested groups of any generation, only effects on adrenals are reported in mid and high dose groups which is probably also related to changes in steroidogenesis.

Therefore, a number of significant endpoints were affected in a statistically significant manner. This was demonstrated by delayed sexual maturation in both male and female offspring (delayed vaginal opening and prepubital separation). It was also observed decreased steroidogenesis (progesterone and 17β-estradiol) in females from 500 ppm, vacuolation of the adrenal cortex apparent in parental animals and offspring, and ovary interstitial vacuolation (1500 ppm), at dose levels not otherwise systemically toxic. They questioned the validity of disregarding statistically significant findings of this nature on the basis that the alterations are within the HCD ranges and therefore not toxicologically relevant.

Assessment and comparison with the classification criteria

RAC Assessment of the reproductive toxicity study

Note

-The units of mg/kg bw/day in food should be replaced with ppm.

-The CLH report in Table 102 gives the NOAEL (development) at 10 mg/kg bw/day based on post-natal adrenal effects (rat 2-generation study). The RAC considers this to be systemic toxicity and 10 mg/kg bw/day to be a systemic NOAEL for offspring. The developmental NOAEL is > 100 mg/kg bw/day.

-Table 103 of the CLH report has points 1 – 5 in the legend which don't seem to apply to this study.

-The introduction table under 4.11.1.1 is inconsistent with Table 102 of the CLH report (Different NOAELs for reproduction). It's also inconsistent with the conclusion (p. 126).

There was no general toxic effect of administration of 0 (control), 150, 500, or 1500 ppm for two successive generations in a well conducted and reported study. There were no changes detected between F0 and F1 parental animals of the treated and control groups in mating indices, pregnancy rates, duration of gestation and male fertility. Likewise, no treatment-related changes were detected in litter size, post-implantation loss, live birth index, viability index, lactation index, sex ratio, clinical signs or pup weight of the F0 offspring (F1 pups) or the F2 pups. In the F2 pups mean body weights were decreased (systemic toxicity from day 7) during lactation at 1500 ppm and anogenital distance was significantly increased (not toxicologically relevant) in males at 150 ($p < 0.01$) and 1500 ($p < 0.05$) ppm. F2 pup mean body weights were not different from controls at birth.

A number of factors were affected by treatment at the 500 ($p < 0.05$) and 1500 ($p < 0.01$) ppm groups which may be considered potentially relevant to reproduction at dose levels causing no other significant toxicity.

1. Significantly increased incidence of white colour of adrenals, increased absolute and relative weights of adrenals and increased diffuse hyperplasia of the zone glomerulosa in F0 and F1 males at 1500 ppm and from 500 ppm in females were noted.
2. The adrenals of F1 and F2 pups from 500 ppm showed significant increases in weights and histopathological findings such as diffuse hypertrophy in zona glomerulosa and/or zona fasciculata. F2 pups in the 1500 ppm group showed white

in colour of the adrenals. These findings in adrenals were consistent with observations in parental animals.

3. Examination of ovaries revealed an increased mean weight (F0 and F1) and significantly higher vacuolation of interstitial cells ($p < 0.1$) in F1 parental females of the 1500 ppm group than controls.
4. The mean oestrous cycle length in the F1 1500 ppm group was slightly but significantly ($p < 0.05$) longer than that in the controls.

   Control: 4.0, high dose group (1500 ppm): 4.2 days

   HCD mean: 4.15; HCD range: 4.0 – 4.3 days
5. Sexual development of F1 parental males: increased mean days for completion of preputial separation at 1500 ppm ($p < 0.01$) while body weight was comparable to control.

   Control: 40.9 ± 1.5, high dose group: 41.9 ± 1.5 days

   HCD mean: 41.76; HCD range: 40.7 – 42.5 days
6. Sexual development in F1 parental females: statistically significant increases in the mean days of age at completion of vaginal opening at 500 ppm ($p < 0.05$) and 1500 ppm ($p < 0.01$), while the mean body weight on the completion day in both of the groups were comparable to that in controls.

   Control: 29.5 ± 2.2, medium dose (500 ppm): 30.7 ± 2.1 and high dose (1500 ppm): 31.0 ± 1.9 days

   HCD mean: 30.8; HCD range: 28.2 – 31.8 days
7. Concentrations of FSH in the 500 and 1500 ($p < 0.05$) ppm groups, progesterone in the 150 ($p < 0.05$), 500 ($p < 0.01$) and 1500 ($p < 0.01$) ppm groups, and 17β-estradiol in the 1500 ($p < 0.05$) ppm were significantly lower in F1 dams than the corresponding values in the control group, respectively.

Table: Summary of maternal and offspring effects

Dose ppm	0		150		500		1500	
	M	F	M	F	M	F	M	F
Maternal								
Organ weights	-	-	-	-	-			
-Adrenals	-	-	-	-	-	↑	↑	↑
-Ovaries	-	-	-	-	-	↑	-	↑
-thyroid	-	-	-	-	-	-	-	↑
Histology								
-hypertrophy, zona glomerulosa	3/23	2/23	2/23	3/23	6/21	0/21	**12/24**	**10/23*** **
-hypertrophy, zona fasciculate	1/23	4/23	0/23	4/23	8/21	4/21	**22/24**	**16/23*** **
-ovary, interstitial cell vacuolation	-		-		-		**-**	
F0		0/24		0/24		0/24		1/24
F1		2/24		2/24		3/24		**14/24*** **
Oestrus cycle length (days)	-		-		-		-	
F0	-	4.0	-	4.0	-	4.0	-	4.1
F1		4.0		4.0		4.0		**4.2***
Steroid hormones (F1 parents)								
-FSH (ng/ml)	-	7.6±0.9	-	9.3±1.1	-	**3.7±2.1***	-	**3.7±1.7***
-progesterone (ng/ml)	-	15.7±4.1		**11±4.7***		**10±3.5** **		**8.3±2.4** **
-17β-estradiol (pg/mL)		19±4.9		18.6±6		17.4±4.8		**12.7±4.9***
Sexual dev. (F1)								
-preputial separation	40.9	-	40.5	-	41.2	-	**41.9***	-
-vaginal opening	-	29.5	-	30.0	-	**30.7***	-	**31.0***
Offspring								
-adrenal weight	-	-	-	-	-	↑	↑	↑
Histology								
-hypertrophy, zona glomerulosa	4/24	1/24	5/24	5/24	**9/24**	7/24	**11/24**	**21/24**
-hypertrophy, zona fasciculata	0/24	6/24	0/24	8/24	0/24	7/24	0/24	**13/24**
-vacuolation, zona fasciculata	3/24	0/24	4/24	0/24	3/24	0/24	**8/24**	0/24
-ovary, interstitial vacuolation	-	2/24	-	2/24	-	3/24	**-**	**14/24**

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.1 ↑ statistically significant in F0 and F1

The clear effects on adrenal weight and adrenal cortex histology in the rat (consistent with the sub-chronic and chronic data) are concurrent with a reduction in the steroid hormones progesterone and 17β-estradiol and could support a mechanism for the observations of delayed sexual development in both male and female F1 animals, i.e., the observed effects can be explained by impaired steroidogenesis. It is acknowledged that the findings are dose-related, statistically significantly different from concurrent controls and most likely treatment related (both in the study and by the RMS). However, it is clear that there is no adverse effect on fertility or reproduction as a consequence and therefore classification is not required as no adverse effect/functional deficit occurred. *RAC notes that the high dose (of 100/140 mg/kg bw/day dietary) may not be high enough as, other than effects on the adrenals, no systemic toxicity was elicited even at the top dose* (whereas the high dose in the developmental toxicity study was 1000 mg/kg bw/day by gavage (York, 2001)). The possibility that more pronounced effects, possibly adverse, could occur at higher doses was not examined. Also, the argument that the various increased values are within the HCD range is less relevant if the effects are considered to be treatment-related. The altered parameters should be compared to the HCD mean not just to its range.

The RMS considers the following as the appropriate adverse levels (LOAELs)

Parental toxicity at 500 ppm based on increased adrenal weight and hypertrophy of adrenal cortical cells. This is in agreement with the conclusion of the DS.

Offspring LOAEL at 500 ppm (35 mg/kg bw/day) based on increased adrenal weights and histopathology in offspring. This was considered a developmental LOAEL by the DS. **RAC does not agree**; pup weights were equivalent to control at birth; these findings are systemic toxicity manifested during lactation and most likely related to ingestion of chow from day 7 onwards. **This is therefore a systemic toxicity LOAEL for offspring.**

Reproductive LOEL > 500 ppm (≈35 mg/kg bw/day) based on no effect on reproduction other than slightly increased oestrus length and reduced steroid hormone levels without adverse effect. Delayed sexual development is considered under the reproduction criteria (CLP Guidance, 2017). Delayed sexual development was seen in F1 males and females at ≥ 500 ppm and was considered treatment-related (but slight and not associated with an adverse outcome).

Although the adrenal (and to some extent the ovaries) are target organs of cyflumetofen, it can be concluded that the effects observed in the 2-generation reproduction study and in the other repeated dose studies, do not lead to *adverse* effects in general, on fertility or its progeny secondary to the changes in endocrine function.

Developmental toxicity

Rat developmental toxicity

A developmental toxicity study was performed in rats in accordance with OECD TG 414. Female rats were mated and administered 0, 50, 250 or 1000 mg/kg bw/day cyflumetofen by gavage (purity 97.67%) from day 6-19 of gestation. The only maternal toxicity seen was reduced body weight gain (77% of control) and increased adrenal weights and vacuolation of adrenal cortical cells from 250 mg/kg bw/day. Reproduction endpoints were unaffected by treatment and no treatment-related malformations occurred. Skeletal examination showed a delayed ossification at 250 and 1000 mg/kg bw/day as shown by incompletely ossified sternal centra. Foetal incidence of wavy ribs was increased ($p < 0.01$) at 1000 mg/kg bw/day, the litter incidence was not increased however. No other gross external, soft tissue or skeletal variations were increased by treatment. In conclusion, some skeletal variations occurred concurrent with effects on maternal adrenal glands from 250 mg/kg bw/day.

Table: Summary of maternal and offspring effects in the rat developmental study

Dose mg/kg bw/day	**0**	**50**	**250**	**1000**
Maternal bw change (net)	27.1±8.8	27.2±9.9	26.2±7.1	20.9±8.4*
Adrenal wt (abs)				
-Right	0.045±0.008	0.047±0.009	**0.049±0.009***	**0.06±0.010****
-Left	0.048±0.008	0.051±0.009	0.055±0.008	**0.064±0.011****
Adrenals (rel)				
-Right	13.0±2.2	13.9±2.0	13.9±2.3	**17.9±2.9****
-Left	13.9±2.0	14.7±2.7	**15.5±2.7***	**18.6±2.9****
Histology				
-hypertrophy, cortical cell, diffuse, bilateral	0	0	0	**14**
-vacuolation, cortical cell, diffuse, bilateral	0	0	2	**24**
Foetal				
Sternal centra: incompletely ossified				
Litter N (%)	4 (16.7)	2 (8.7)	**9 (39)****	**9 (36)***
Foetal N (%)	5 (3.3)	4 (2.7)	13 (7.9)	13 (8.3)
HCD: 17 studies June 1998-June 2000, 406 litters, 3051 foetuses				
Litter; 50 (12.32%), 1 – 5 (4.2% - 21.7%). Foetal; 61 (2%), 1 – 7 (0.6% – 4.0%)				

Ribs: Wavy Litter N (%) Foetal N (%) *HCD:* *Litter; 5 (1.23%), 0-1 (0 – 4.8%). Foetal; 5 (0.16), 0-1 (0 – 0.6%)*	 5 (21) 6 (3.9)	 3 (13) 5 (3.4)	 4 (17) 9 (5.5)	 7 (28) **16 (10)****

*p≤0.05; ** p≤0.01

Rabbit developmental toxicity

A developmental toxicity study was performed in rabbits in accordance with OECD TG 414. Female rabbits were mated and administered 0, 50, 250 or 1000 mg/kg bw/day cyflumetofen by gavage (purity 97.67%) during days 6-28 of gestation. Absolute liver weights were decreased from 250 mg/kg bw/day without statistical significance (7.8% and 8.3%, respectively) and adrenal weights were reduced at 1000 mg/kg bw/day (right 11.9% and left 6.3%). Body weight gains were reduced for the entire gestation period from 250 mg/kg bw/day (24.3% and 32.4%, respectively), also without statistical significance, concurrent with reduced food consumption.

A number of foetal parameters were altered at 1000 mg/kg bw/day. Reduced foetal body weights were associated with significantly increased incompletely ossified 1st sternal centre and significantly increased litter incidences of angulated hyoid alae. All are commonly occurring variations which can be increased in incidence with reduced foetal weights.

Table: Summary of maternal and offspring effects in the rabbit developmental study

Dose (mg/kg bw/day)	**0**	**50**	**250**	**1000**
Maternal weight gain				
6-9	+0.01	+0.00	-0.02	-0.03
6-29	+0.37	+0.34	**+0.28 (-24.3%)**	**+0.25 (-32.4%)**
6-29 (Corrected for gravid uterus)	-0.17	-0.17	**-0.23 (-35%)**	**-0.26 (-53%)**
Foetal weight (g/litter)	46.96 ±5	45.56±5.5	45.4±6.2	**41.1±4.4****
Skeletal findings *Litter incidence:* - incompletely ossified sternal centra *Foetal incidence:* - incompletely ossified sternal centra	 0 0	 0 0	 1 (4.5%) 1 (0.6%)	 **8 (32%)**** **16 (7.3%)****
HCD: 67 studies Jan 1999-2001, 885 pregnant dams at sectioning *Litters; mean 14 (1.89%), range 0-2 (0-10.5%) in 11/44 studies. Foetal; mean: 15 (0.24), range 0-2 (0-1.2%)*				
Litter incidence: - hyoid, angulated alae *Foetal incidence* - hyoid, angulated alae	2 (8.3%) 3 (1.6%)	2 (8.3%) 3 (1.5%)	2 (8.3%) 3 (1.7%)	**10 (40%)**** **11 (5%)**
In house HCD (Jan 1999-2001) *Litters; mean 94 (12.70%), range 0-6 (0-31.6%) in 36/44 studies. Foetus; mean 111 (1.76%), range 0-10 (0-6.4%)*				

Comparison with the criteria

Sexual function and fertility

Arguments for classification

There were a number of treatment-related effects on reproduction associated parameters which are relevant to classification, as described above. All effects were statistically significant and a treatment and dose-relationship was apparent. A plausible mechanism based on a targeted effect on lipid metabolism and steroidogenesis is supported by the pattern of effects. Comparison to the HCD shows that many endpoints are slightly above the mean value but within the range of

values recorded. It is noted that where the effects are believed to be treatment related, a comparison to HCD is less relevant. It is further noted that the maximum dose in the rat 2-generation study was limited to about 140 mg/kg bw/day. More pronounced effects may have been observed if the doses were greater than those tested.

Argument against classification

Even though a plausible mechanism could be supported, the effects were relatively slight at the maximum dose tested. Most importantly, there was no functional deficit on fertility or reproduction in either generation. There is no clear evidence of an adverse effect on sexual function and **Cat. 1B is not supported**.

The study was well conducted and no deficiencies were detected, however the choice of dose levels may be questioned. In this study, rats were only dosed up to a maximum of 140 mg/kg bw/day. It is not clear why higher doses were not tested, especially since much higher doses of 250 – 1000 mg/kg bw/day were tolerated by rodents and rabbits in the developmental studies. There is thus a lack of sufficient evidence to support classification. As the effects seen do not impact on fertility or reproductive function, **Cat. 2 is not supported**.

Developmental toxicity

Arguments for classification/non-classification

In the rat study, some delayed ossification and increased incidence of wavy ribs occurred concurrent with significantly reduced maternal weight gain and effects on maternal adrenal glands from 250 mg/kg bw/day. There were no increases in malformations or other adverse developmental findings. **Classification in Cat. 1B is not supported by this study**.

In the rabbit study, some effects on maternal weight gain and adrenal weight were seen from 250 mg/kg bw/day (probably treatment-related but not statistically significant). At the high dose level, foetal weight was significantly reduced (≈10%) in conjunction with reduced ossification and an increase in hyoid alae change with no increase in malformations. Reduced foetal weight in one of two species tested is **not sufficient evidence of adversity to classify in Cat. 1B**.

In both species there was an increase in commonly occurring skeletal variations at the high dose causing some maternal toxicity. In addition, foetal weight was decreased in the rabbit. These findings were considered for Cat. 2. However, it must be noted that there were no increases in malformations in either of the two species tested. In conclusion, an increase in commonly occurring skeletal variations were observed. Although treatment-related, these effects are not considered by themselves sufficient to meet the criteria for adversity, they are not considered permanent developmental effects and classification in Cat. 2 is not supported.

In conclusion, RAC agrees with the DS that **classification is not warranted for sexual function and fertility or for development of the offspring**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Cyflumetofen is a new active substance for plant production products (specifically an acaricide used in both indoor and outdoor spray applications on ornamental crops, nursery trees, perennial ornamentals and public greens for the control of *Tetranychyus urticae* (red spider mite)). It was approved under Regulation (EC) No 1107/2009 via Commission Implementing Regulation (EU)

No 22/2013 and subsequently amending the Annex to Commission Implementing Regulation (EU) No 540/2011. The substance currently has no harmonised classification. The Dossier Submitter proposed not to classify the substance as hazardous to the aquatic environment.

Degradation

A hydrolysis study according to JMAFF, EPA N:161-1, EU 95/36/EC, FAO (1993) and in compliance with GLP was run at pH 4, 5, 7 and 9 at 25 °C and at a concentration of 0.1 mg/L. Cyflumetofen was susceptible to hydrolysis in acidic, neutral and basic buffer solutions, the hydrolysis rate increasing with increasing pH. Hydrolytic half-lives of cyflumetofen at 25 °C are 7.7 d (pH 4), 6.0 d (pH 5), 9.8 hrs (pH 7) and 10.3 min (pH 9). At environmentally relevant pH values (pH 5-7), five hydrolysis products exceeding 10% of applied radioactivity were identified (A-1, A-2, A-18, AB-1 and B-1).

The photodegradation of radio-labelled cyflumetofen in water was studied according to JMAFF, EPA N:161-2, EU 95/36/EC, FAO (1993). The study, in compliance with GLP, was carried out at 25 °C with continuous artificial light. The half-life was 1.28h at pH 5 (aqueous buffer solution) and 1.07 h in natural water, indicating that cyflumetofen is susceptible to aquatic photolysis. Three degradation products which account for > 10% were identified in aqueous buffer solution (B-1, AB-7 and AB-15), in natural water AB-1 was also observed in significant amounts.

There is no screening test for biodegradability available.

Two water/sediment studies were performed with a ^{14}C label in either of the two aromatic rings of cyflumetofen (A-ring and B-ring). These studies, carried out according to OECD TG 308 and in compliance with GLP, used the same two water/sediment systems; the first one was run for 57 and 98 days, the second one for 103 days. Both studies showed that cyflumetofen partitions from the water phase to the sediment and exhibited very low to moderate retention in the whole system (DT_{50} values range from 0.08 to 14 days). Mineralisation to CO_2 was a minor process (2-3%) except in the A-label study where 20% mineralisation was observed in one of the two test systems. Bound residues accounted for between 13 to 33% AR. Several relevant degradation products (>10% of applied radioactivity) were formed in both compartments (AB-11, B-1) or in the water compartment only (A-2, Met-1, Met-8) and in the sediment compartment only (AB-1, Met-4). Metabolite B-2 was formed in the water/sediment study with B-radiolabelled cyflumetofen only. Two metabolites were not identified (Met-4 and Met-8). Where possible, DT_{50} system values were calculated for metabolites (range from 0.77 to 320 days).

Based on the results of the simulation tests, the dossier submitter considered cyflumetofen as not rapidly degradable since it appeared susceptible for primary degradation but not ultimate mineralisation. Due to missing information for all degradation products, it could not be demonstrated that they do not fulfil the criteria for classification as hazardous to the aquatic environment.

Bioaccumulation

A fish bioconcentration study was available for cyflumetofen, carried out according to OECD TG 305 and in compliance with GLP. Carp (*Cyprinus carpio*) were exposed to B-[ring-U-^{14}C] cyflumetofen for 21 days in a flow-through system, followed by 32 days of depuration in clean water. Nominal concentrations of 1.0 and 10 µg/L, plus solvent control were tested in one replicate aquarium containing 48 fish at test initiation (22 fish were used for the solvent-control).

BCF values for total radioactivity in whole fish were 170 and 146 L/kg wwt at 1.0 and 10 µg a.s./L respectively (lipid BCF normalised to 1% fat 34 and 29 L/kg wwt). Since cyflumetofen was not detected in any fish sample, the BCF for cyflumetofen was < 100 L/kg at both exposure levels.

No measured bioaccumulation data are available for the seven major metabolites identified to be relevant for surface water (A-2, B-1, B-2, AB-11, AB-15, Met-1 and Met-8). For some of them

(B-2, AB-7, AB-11), despite the estimated Log K_{OW} values indicate a potential for bioaccumulation, the bioconcentration was not considered to be of concern due to their short half-lives in water. No assessment was made for unidentified metabolites Met-1 and Met-8.

Aquatic toxicity

Summary of relevant information on aquatic toxicity performed with cyflumetofen and for three of the relevant aquatic degradation products AB-11, B-1 and B-2:

Method	Substance tested	Purity [%]	Species	System	Endpoint	Value [a] [mg/L]	Remarks
Acute toxicity to fish							
ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Oncorhynchys mykiss* (rainbow trout)	96h (flow-through)	Survival, LC_{50}	>0.63 (mm)	Migchielsen, 2003a, (IIA 8.2.1.1/01) [d]
ISO 7346-3; EEC C.1; OECD 203	cyflumetofen	98.0%	*Cyprinus carpio* (carp)	96h (flow-through)	Survival, LC_{50}	>0.54 (mm)	Migchielsen, 2003b (IIA 8.2.1.1/02) [d]
Acute toxicity to invertebrates							
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.0%	*Daphnia magna*	48h (flow-through)	Immobility, EC_{50}	>0.063 (mm)	Bouwman, 2003a (IIA 8.3.1.1/01) [d]
ISO 6341; EEC C.2; OECD 202	cyflumetofen	98.4%	*Daphnia magna*	48 h (flow-through)	Immobility, EC_{50}	0.70 (im) [f]	Migchielsen 2009b (IIA 8.3.1.1/02) [d]
ISO 6341; EEC C.2; OECD 202	AB-11	99.6%	*Daphnia magna*	48h (static)	Immobility, EC_{50}	>0.5 (nom) or >0.476 (mm)	Migchielsen, 2009a (IIA 8.3.1.1/03) [d]
ISO 6341; EEC C.2; OECD 202	B-1	99.99%	*Daphnia magna*	48h (static)	Immobility, EC_{50}	>180 (nom)	Migchielsen, 2008b (IIA 8.3.1.1/04) [d]
ISO 6341; EEC C.2; OECD 202	B-2	99.9%	*Daphnia magna*	48h (static)	Immobility, EC_{50}	>0.039 (im) or >0.0062 (mm)	Bouwman, (2009a) IIA 8.3.1.1/05)
Acute toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate, EC_{50}	>0.30 (im) or >0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/ growth rate, EC_{50}	>0.5 (nom) or >0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneriella subcapitata*	96h (static)	Yield/ growth rate, EC_{50}	>100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/ growth rate, EC_{50}	>0.073 (im) or >0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]
Chronic toxicity to fish							
OECD 215	cyflumetofen	98.4%	*Cyprinus carpio* (carp)	28d (flow-through)	Survival/growth, NOEC	0.072 (mm)	Migchielsen, 2007a (IIA 8.2.3/01) [d]

OECD 212	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	8d (flow-through)	Survival/hatching, NOEC	≥ 0.145 (mm)	Migchielsen, 2008a (IIA 8.2.4/01) [d]
OECD 210	cyflumetofen	98.4%	*Pimephales promelas* (fathead minnow)	31d (flow-through)	Hatching/ larval survival/growth, NOEC	0.054 (mm)	Migchielsen, 2010 [e]
Chronic toxicity to invertebrates							
OECD 211; ISO International Standard 10706:2000 (2000-03-30)	cyflumetofen	98.4%	*Daphnia magna*	21d (flow-through	Mortality, NOEC	0.065 (mm) [b]	Migchielsen, 2007b (IIA 8.3.2.1/01) [d]
Chronic toxicity to algae							
ISO 8692; EEC C.3; OECD 201	cyflumetofen	98.0%	*Selenastrum capricornutum*	72h (static)	Biomass/growth rate,	>0.30 (im) or >0.0396 (mm)	Bouwman, 2003b (IIA 8.4/01) [d]
ISO 8692; EEC C.3; OECD 201	AB-11	99.6%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/ growth rate, NOEC [c]	>0.5 (nom) or >0.157 (mm)	Migchielsen, 2009b (IIA 8.4/02) [d]
ISO 8692; EEC C.3; OECD 201	B-1	99.99%	*Pseudokirchneriella subcapitata*	96h (static)	Yield/ growth rate, NOEC [c]	>100 (nom)	Migchielsen, 2008c (IIA 8.4/03) [d]
ISO 8692; EEC C.3; OECD 201	B-2	99.9%	*Pseudokirchneriella subcapitata*	72h (static)	Yield/ growth rate, NOEC [c]	>0.073 (im) or >0.0101 (mm)	Bouwman, 2009b (IIA 8.4/04) [d]
Sediment dwelling organisms							
OECD 219	cyflumetofen	98.4%	*Chironomus riparius*	28d (static)	Emergence/development (water spiked) , NOEC	≥ 0.064 (im)	Desmares-Koopmans, 2009a (IIA 8.5.2/01) [d]
OECD 218	AB-1	99.8%	*Chironomus riparius*	28d (static)	Emergence/development (sediment spiked) , NOEC	59.6 mg/kg (im)	Desmares-Koopmans, 2009b (IIA 8.5.2/02) [d]

[a](mm): mean measured concentrations; (nom): nominal; (im): initially measured

[b] This study is less reliable due to high mortality in the control. However, the experts in the Pesticides Peer Review Expert Meeting considered that a new chronic study with daphnids for cyflometofen is not required based on the following arguments: in the study, no effects were seen on reproduction (thus: NOECreproduction ≥151 µg a.s./L); the chronic NOEC for daphnids of 65 µg a.s./L is based on mortality which is a worst case approach; the NOEC of 65 µg a.s./L is comparable to the acute NOEC for daphnids.

[c] The NOEC was taken from the original study report as this endpoint was not reported in the EFSA evaluation and the DAR.

[d] As summarised in the Draft Assessment Report prepared in the context of the possible inclusion of cyflumetofen in Annex I of Council Directive 91/414/EEC, Revised Volume 3, Annex B.9; September 2011.

[e] This study (Migchielsen, 2010: Fish early-life stage toxicity test with OK-5101 (flow-through), NOTOX B.V., 's-Hertogenbosch, The Netherlands. Unpublished report No. 487052) was submitted as supplement to the DAR. Note:"OK-5101" is used as code for "cyflumetofen".

Toxicity to Fish

The acute toxicity of cyflumetofen (purity 98.0%) for *Oncorhynchys mykiss* (rainbow trout) and *Cyprinus carpio* (Common carp) was tested in a 96h flow-through study in accordance with ISO 7346-3; EEC C.1; OECD TG Guideline 203, (Migchielsen, 2003a and b). Juvenile organisms were exposed to a nominal concentration of 1.0 mg of the test substance/L, using acetone as solvent carrier. The mean measured concentrations was 0.63 mg cyflumetofen/L for the rainbow trout test, and 0.54 mg cyflumetofen/L for the carp test, in both cases exceeding the water solubility of cyflumetofen (reported to be 0.028 mg/L). During the tests, small amounts of precipitate and a thin floating layer were observed in the solution with cyflumetofen but this was reported not to have affected the fish. Symptoms of toxicity were noted in rainbow trout exposed to cyflumetofen within 24 hours of exposure and included slower swimming and discolouration. The LC_{50} was > 0.63 mg/L. No mortality or symptoms of toxicity were noted in the carp exposed to cyflumetofen. The LC_{50} was > 0.54 mg/L.

For the chronic toxicity of cyflumetofen, three studies of Migchielsen, 2007a, 2008a and 2010 (see table above) were reported. RAC notes that the Migchielsen, 2007a and 2008a studies are extracted from the revised DAR report (Sep. 2011), whilst the most recent data provided (Migchielsen, 2010) refers to a supplement document to the DAR, not currently available to RAC.

In the juvenile growth test with carp (Migchielsen, 2007a, OECD TG 215) the nominal concentrations were 10, 22, 46, 100 and 220 µg/L in a flow-through test design for 28 days. Mean measured concentrations in the test were 7.2, 16, 34, 72 and 179 µg/L. Increases in body weight, growth rate and body length were significantly different from control (at 5% level, according to DAR, 2011) at the highest mean measured concentration of 179 µg/L, above the water solubility of cyflumetofen. Therefore, the related NOEC was 0.072 mg/L (mean measured concentration).

In the Migchielsen (2008a) test (OECD TG 212), fathead minnow was exposed at nominal concentrations of 10, 46 and 220 µg/L in a flow-through test design for 8 days. Mean measured concentrations in the test were 5.9, 27 and 145 µg/L. The only observed effect was for the larval survival at the highest mean measured concentration 145 µg/L, but the slight reduction was not statistically significant (see section "additional information"). Therefore, the NOEC was ≥ 145 µg/L.

The early life stage toxicity test in fathead minnow (Migchielsen, 2010, OECD TG 210) showed no effects on hatching success, larval survival, body length and body weight at any tested concentration (nominal) of 15, 30 and 60 µg/L in a flow-through test design for 31 days. Mean measured concentrations were 11, 34 and 54 µg/L. Therefore, the NOEC was the highest tested concentration of 54 µg/L (mean measured), exceeding the water solubility.

No information is available for the metabolites AB-11 and B-2 and Metabolite B-1.

Toxicity to Aquatic invertebrates

Two acute toxicity tests of cyflumetofen for *Daphnia magna* and three for the main metabolites were reported (see table above). All tests were according to OECD TG 202 without any toxic effects up to the water solubility levels.

For the chronic toxicity of cyflumetofen, juvenile *Daphnia magna* (Migchielsen, 2007b) were exposed to cyflumetofen at nominal concentrations of 10, 22, 46, 100 and 220 µg/L in a flow-through test for 21 days, in accordance with ISO and OECD TG 211 guidelines. Mean measured concentrations in the test were 7.5, 12, 32, 65 and 151 µg/L, respectively. Mortality at 151 µg/L

was significantly different from that in the solvent-control after 20 days. Parental body length and reproduction was not affected at any concentration. Under flow-through conditions, the 21-day NOEC of cyflumetofen to *Daphnia magna* was 65 µg/L, based on a significant effect on parental survival after 20 days.

The mortality in the blank control (58%) and in the solvent control (28%), of the study was above the validity criterion of the guideline of ≤ 20%. Since the study does not fulfil the validity criterion of the relevant guidelines, the reliability of the study was discussed by the experts in the Pesticides Peer Review Expert Meeting. It was concluded that: […] *in the study, no effects were seen on reproduction (thus: $NOEC_{reproduction}$ ≥ 151 µg/L); the chronic NOEC for daphnids of 65 µg/L is based on mortality which is a worst case approach; the NOEC of 65 µg/L is comparable to the acute NOEC for daphnids*.

Toxicity to algae

Cyflumetofen (purity 98.0%) was tested for 72 hours under static conditions on the green algae *Selenastrum capricornutum*, in accordance with ISO 8692; EEC C.3; OECD TG 201 (Bouwman, 2003b). The substance was dissolved in acetone because of the low water solubility (i.e. 0.028 mg/L). The initial measured concentration of 0.30 mg/L exceeded the water solubility of cyflumetofen. The mean measured concentration in the test, calculated with geometric means, was 0.0396 mg/L. Cyflumetofen did not reduce algal growth rate so the 72 h ErC_{50} values were > 0.30 mg/L (initially measured) or > 0.0396 mg/L (mean measured). The NOEC for growth rate was > 0.30 mg/L (initially measured) or > 0.0396 mg/L (mean measured).

The three metabolites AB-11, B-1 and B-2 were tested on *Pseudokirchneriella subcapitata* and the studies were performed in accordance with ISO 8692; EEC C.3; OECD TG 201.

The green algae was exposed for 72 hours to the metabolite AB-11 (purity 99.6%) dissolved in acetone (Migchielsen, 2009b). No reduction in algal growth rate was observed, and the following values were established: ErC_{50} > 0.493 mg/L (initially measured) or > 0.157 mg/L (mean measured); NOEC for growth rate were > 0.493 mg/L (initially measured) or > 0.157 mg/L (mean measured).

For the metabolite B-1, the nominal test concentrations of 0.1, 1.0, 10 and 100 mg/L were used, the measured concentrations were in agreement with them (Migchielsen, 2008c). The test was performed for 96 hours under static condition and no reduction in algal growth rate was observed at the higher concentration. The 72 h ErC_{50} value for B-1 was > 100 mg/L; the NOEC for yield and growth rate was > 100 mg/L.

Pseudokirchenriella subcapitata, (Bouwman, 2009b) was exposed to B-2 (purity 99.9%) for 72 hours under static conditions; acetone was used as a solvent carrier. B-2 did not reduce algal growth rate, the 72 h ErC_{50} values were > 0.073 mg/L (initially measured) or > 0.0101 mg/L (mean measured). The NOEC for growth rate were > 0.073 mg/L (initially measured) or > 0.0101 µg/L (mean measured).

Comments received during public consultation

Three MSCAs and two stakeholders commented on ENV classification. Only one MS did not agree with the proposal of no classification for environmental hazards and proposed classification and labelling for long-term aquatic hazard with category Chronic 1; H410.

The reasoning for the disagreement was the presence of a chronic toxicity study on fish (Salinas, 2001, as reported in EFSA Journal 2016; 14(11):4635) which reported a NOEC value of 0.0292 mg a.s./L (mean measured). This value was considered by the MSCA as equal to the water

solubility of 0.028 mg/L (at 20°C and pH 7), proposing a classification for long-term aquatic hazard as Aquatic Chronic 1; H410.

According to the Dossier Submitter's (DS) response, the Salinas (2011) study is relevant and valid, but it does not change the proposed no classification for chronic toxicity. Indeed, the DS pointed out that the toxicity value reported in the EFSA Journal was ≥ 0.0292 mg/L because no effects (to hatching success, survival or growth of the fathead minnow) were observed at the tested concentration which should be regarded as close to the water solubility limit (EFSA Journal, 2016). Water solubility is reported as 28 µg/L at 20 °C and pH 7.

Moreover, the DS reported that "EFSA *considers it more appropriate to remove the measured concentrations which exceeded the water solubility in the calculation of the exposure value. However, this does not affect the overall conclusion of the study i.e. that there were no effects at the tested concentration which should be regarded as close to the water saturation level."*

One MS commented on the acute toxicity test of metabolite B2 to *Daphnia magna* (Bouwman, 2009a) saying that for rapidly hydrolysing substances it is considered justified by the DS to base the toxicity endpoint on the measured initial concentration. Referring to the CLP guideline (I.4.1) recommendation, the commenting MSCA reminded that for unstable substances, the geometric mean concentration at the start and the end of test for classification purposes should be considered. RAC noted that for the metabolite B-2, the concentration of the start was 0.039 mg/L and at the end of test the concentration resulted below the analytical detection limit (0.002 mg/L). Therefore, according to CLP guideline Version 5.0 – July 2017 (I.4.1) the $L(E)C_{50}$, should be calculated based on the geometric mean concentration and half of the detection limit. The result is = 0.0062 mg/L, as reported in the DAR (Sept. 2011) and in the CLH report (Table 147).

Assessment and comparison with the classification criteria

Degradation

RAC agreed with the DS proposal to consider cyflumetofen as not rapidly degradable. The substance is not hydrolytically and photochemically stable, data on ready biodegradability are not available and the results of water/sediment simulation studies showed that cyflumetofen is susceptible for primary degradation (DT_{50} < 16 days), but it did not ultimately degrade to a level greater than 70% within 28 days. Annex I, section 4 of the CLP regulation states that rapid degradation can be demonstrated by ready biodegradability or ether evidence of rapid degradation in the environment (≥ 70% abiotic or biotic degradation within 28 days). Furthermore, it states that primary degradation does not normally suffice in the assessment of rapid degradability unless it can be demonstrated that the degradation products do not fulfil the criteria for classification as hazardous to the aquatic environment.

For cyflumetofen, even if some of the relevant metabolites formed in the water-phase can be considered not hazardous to the aquatic environment, the lack of data regarding the unidentified metabolites are sufficient to conclude that cyflumetofen is not rapidly degradable.

Bioaccumulation

The measured BCF (for total radioactivity) in whole fish of 170 – 146 L/kg wwt is below the CLP criterion (BCF ≥ 500). Therefore, RAC agrees with the DS that the bioaccumulation potential of cyflumetofen is low.

Acute aquatic hazards

Acute aquatic toxicity data is available for all three trophic levels. The water solubility of cyflumetofen is 0.028 mg/L (at 20°C and pH 7). No effects on aquatic organisms were observed at test concentrations below or equal to the water solubility limit of cyflumetofen.

Therefore, RAC agrees with the DS's proposal **not to classify cyflumetofen for acute aquatic hazards**.

Chronic aquatic hazards

Chronic aquatic toxicity data is available for all trophic levels and the lowest NOEC value provided by the DS is 0.0396 mg/L (algae; mean measured concentration).

The available information shows no toxicity below or equal to the water solubility limit, therefore the NOEC for classification purposes was considered greater than the measured water solubility (0.028 mg/L at 20°C and pH 7). Although cyflumetofen is considered not rapidly degradable, the experimental BCF value does not exceed the CLP criterion threshold of 500 L/kg, so classification as Aquatic Chronic 4 is not warranted.

Therefore, RAC agrees with the DS's proposal **not to classify cyflumetofen for chronic aquatic hazards**.

Additional references

EFSA (European Food Safety Authority), 2016. Conclusion on the peer review of the pesticide risk assessment for the active substance cyflumetofen in light of confirmatory data. EFSA Journal 2016;14(12):4635, 20 pp. doi:10.2903/j.efsa.2016.4635

EFSA (European Food Safety Authority), 2016. Technical report on the outcome of the consultation with Member States, the applicant and EFSA on the pesticide risk assessment for cyflumetofen in light of confirmatory data. EFSA supporting publication 2016:EN-997. 25 pp.

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)