Committee for Risk Assessment

RAC

Annex 1

Background document

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

bifenthrin

ECHA/RAC/ CLH-O-0000001740-81-01/A1

bifenthrin

EC number: not allocated
CAS number: 82657-04-3

Adopted

24 May 2011

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

In this CLH dossier and according to the CAS entry Bifenthrin is defined as solely the cis-Z-isomer pair (ratio of (1R,3R):(1S,3S) is 50:50); whereas the literature defines Bifenthrin as a combination of cis-isomers and trans-isomers (ratio 97:3) (BCPC & The Royal Society of Chemistry, 1994).

According to an FAO report: (http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/JMPR/Report09/bifenthrin.pdf), "Bifenthrin is the International Organization for Standardization (ISO) approved name for 2-methyl-3- phenylphenyl) methyl (1RS, 3RS)-3-[(Z)-2-chloro-3, 3, 3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropane-1-carboxylate (International Union of Pure and Applied Chemistry [IUPAC]), for which the Chemical Abstracts Service (CAS) No. is 82657-04-3".

Substance Name:	bifenthrin
EC Number:	not allocated
CAS number:	82657-04-3
Registration number (s):	not applicable
Purity:	> 911 g/kg
Impurities:	This information is confidential and then provided in the confidential part of the dossier provided in appendix 1.

Proposed classification based on CLP criteria:

Carc. 2 – H351
Acute Tox. 3 – H331
Acute Tox. 2 – H300
STOT RE 1 – H372 (nervous system)
Skin Sens. 1 – H317 ; 2nd ATP: subcategory 1B
Aquatic Acute 1– H400
Aquatic Chronic 1 – H410

Proposed classification based on Directive 67/548/EEC criteria:

Carc. Cat. 3; R40
T; R23/25
Xn; R48/22
R43
N; R50/53

Proposed labelling:

Based on CLP :

Pictograms:	GHS06, GHS08, GHS09
Signal word:	Dgr
Hazard statements:	H351 – H331 –H300-H317 – H-372 - H410

Based on Directive 67/548/EEC:

Indications of danger:	T, N
Risk phrases:	R23/25; R40; R43; R48/22; R50/53
Safety phrases:	S23, S24, S36/37, S38, S45, S60, S61

Proposed specific concentration limits (SCL):

Under CLP (2nd ATP), an M-factor = 10 000 would apply for Aquatic Acute 1 (H400), while an M-Factor = 100 000 would apply for Aquatic Chronic 1 (H410) classification.

Under Directive 67/548/EEC, SCL corresponding to an M-factor = 10 000 under CLP are proposed for environment:

Specific concentration limits:

$C \geq 0.0025$ %	N; R50/53
$0.00025\ \% \leq C < 0.0025\ \%$	N; R51/53
$0.000025\ \% \leq C < 0.00025\ \%$	R52/53

Proposed notes (if any):

None

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

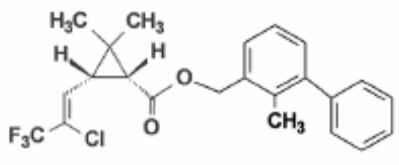
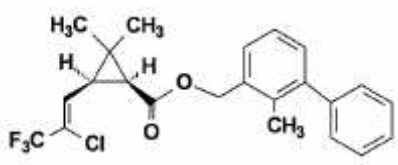
Chemical Name:	Bifenthrin
EC Name:	Not allocated
CAS Number:	82657-04-3
IUPAC Name:	2-methylbiphenyl-3-ylmethyl (1RS)-cis-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate

1.2 Composition of the substance

Bifenthrin has 3 sites of isomerism and can therefore exist as 8 potential enantiomers. Each of the four pairs of enantiomers is present as racemic mixture. The cis-Z isomers are the predominant species comprising minimum 98% total Bifenthrin (see structure below). The concentration of other isomers is presented in the confidential part (appendix 1).

Chemical Name:	Bifenthrin
EC Number:	Not allocated
CAS Number:	82657-04-3
IUPAC Name:	2-methylbiphenyl-3-ylmethyl(1RS)-cis-3-[(Z)-2-chloro-3,3,3-trifluoroprop-1-enyl]-2,2-dimethylcyclopropanecarboxylate
CAS Name:	(2-methyl[1,1'-biphenyl]-3-ylmethyl (1R,3R)-rel-3-[(1Z)-2-chloro-3,3,3-trifluoro-1-propenyl]-2,2-dimethylcyclopropanecarboxylate
Related CAS information:	CAS#: 439680-76-9 ((1R-cis-Bifenthrin) CAS#: 439680-77-0 (1S-cis-Bifenthrin)
Molecular Formula:	$C_{23}H_{22}ClF_3O_2$

Structural Formula

Z-isomers, min. 98% (ratio 1:1)	
Z-(1R,3R)	Z –(1S,3S)
	

Molecular Weight:	422.88
Typical concentration (%	≥91.1%

w/w):	
Concentration range (% w/w):	no information

1.3 Physico-chemical properties

Table 4.1.11: Summary of physico- chemical properties

REACH ref Annex, §	**Property**	**IUCLID section**	**Purity/Specification**	**Value**	**[enter comment/reference or delete column]**
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	Purified bifenthrin (96.1%)	Waxy beige solid	Spruit W.E.T., et al. 2002
VII, 7.2	Melting/freezing point	4.2	Purified bifenthrin (96.1%)	66.6 - 69.0 °C	Spruit W.E.T., et al. 2002
VII, 7.3	Boiling point	4.3	Purified bifenthrin (96.1%)	Decomposition at 285°C before boiling.	Spruit W.E.T., et al. 2002
VII, 7.4	Relative density	4.4 density	Purified bifenthrin (96.1%)	1.316 g cm³ at 24°C	Spruit W.E.T., et al. 2002
VII, 7.5	Vapour pressure	4.6	Purified bifenthrin (96.5%)	2.431 10^{-5} Pa at 25°C	Hu, H.C., 1983
VII, 7.7	Water solubility	4.8	Purified bifenthrin (97.8%)	< 1 µg/l (pH 4.05) at 20°C < 1 µg/l (pH 7.04) at 20°C 3.76 µg/l (pH 9.22) at 20°C	Françon B. & D. Zenide, 1999
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7 partition coefficient	Purified bifenthrin (96.5%)	log P > 6	Herbst R.M., 1983a
VII, 7.9	Flash point	4.11	Bifenthrin technical (94.93%)	Higher than 110°C	Spruit W.E.T., et al. 2002
VII, 7.10	Flammability	4.13	Bifenthrin technical (94.93%)	No pyrophoric properties	Spruit W.E.T., et al. 2002
VII, 7.11	Explosive properties	4.14	Bifenthrin technical (94.93%)	No explosive properties	Spruit W.E.T., et al. 2002
VII, 7.13	Oxidising properties	4.15	Bifenthrin technical (94.93%)	No oxidizing properties	Spruit W.E.T., et al. 2002
	Auto flammability	4.12	Bifenthrin technical (94.93%)	Not auto-flammable	Spruit W.E.T., et al. 2002
	Thermal stability	4.19	Bifenthrin technical (94.93%)	Not thermally stable in the sense of OECD 113	Spruit W.E.T., et al. 2002
	Solubility in organic solvents	4.9	Bifenthrin technical (94.93%)	Methanol = 48.0g/L Xylen= 556.3g/L Acetone = 735.7g/L N heptane = 144.5g/L Ethyl acetate = 579.8g/L 1,2 dichloroethane = 743.2g/L temperature: 20°C	Spruit W.E.T., et al. 2002

The purity indicated for the physico-chemical properties corresponds to the total bifenthrin. In order to obtain the purity of the substance (cis-Z isomers), a factor of 98% should be applied to the values indicated in the table.

2 MANUFACTURE AND USES

Not relevant for a classification and labeling report.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

The substance is not currently classified in annex I of Directive 67/548/EEC or in Annex VI of CLP regulation.

3.2 Self-classification(s)

A classification Xn; R20, T; R25, R43, N; R50/53 was first proposed by the industry in the scope of the Biocidal Product Directive (98/8/CE).

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 Stability

Hydrolysis in water

A hydrolysis experiment (not under GLP) carried out in buffers with pH 5, pH 7 and pH 9 at 25°C and on test material with a purity of 96.5% showed that bifenthrin is **hydrolytically stable** in water. At initial test concentrations of 0.52 and 5.22 mg/L the DT_{50} value was >22 d at each pH tested (Herbst, 1983). This study suffers several deficiencies, mainly as regards the high concentrations tested, which are higher than the solubility of the substance. Repetition was not deemed necessary however since hydrolysis does not appear to be a major degradation pathway. This is confirmed by the relative stability of bifenthrin in simulation tests (soil, water/sediment).

Photolysis in water

Two photolysis studies are available. In the first photolysis experiment (not under GLP), bifenthrin (purity of test material: 96.6%) is degraded under artificial sunlight with half-life of 11.9 days (irradiation). The main degradation product is the TFP acid (3-2(chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethylcyclopropane carboxylate - max. 38.4%, 14d). Under natural late summer outdoor sunlight irradiation at 41° N (USA) photolysis of bifenthrin was slow with a half-life of about 255 days. No photoproduct accounted for more than 3% (Wu, 1986).

The second photolysis experiment (GLP; following the draft OECD guideline, 2000), performed under artificial lighting and on test material with a purity > 95%, shows that bifenthrin degrades in water under influence of light with irradiation half-life of ca 10 days (24.4 d under sunlight conditions comparable to natural sunlight of the first experiment) (Curry, 2006). Metabolites formed are biphenyl alcohol, biphenyl acid and 4'-OH-bifenthrin. In both studies, results obtained with artificial sunlight are comparable, however, the second study does not explain the differences observed between natural and artificial conditions in the first study.

However, since the photodegradation was not observed under natural sunlight or in presence of sensitizer, **photodegradation is expected to be limited** in natural water.

4.1.2 Biodegradation

4.1.2.1 Biodegradation estimation

4.1.2.2 Screening tests

Ready biodegradation of bifenthrin was firstly investigated according to OECD 301b "Modified Sturm Test" (not under GLP; reliability = 1) and on test material with a purity of 94%. A 11% and 4% degradation was observed within 28 days at test substance concentrations of 10 and 20 $mg.L^{-1}$, respectively. Result from control substance (Sodium benzoate) attained 92% degradation after 28 days and thereby confirming the suitability of the *inoculum* and test conditions (Handley and Horton, 1991).

Therefore, bifenthrin is clearly **not readily biodegradable** in the strict terms of OECD 301b guideline.

4.1.2.3 Simulation tests

Biodegradation in water/sediments systems

A study under GLP was carried out according to OECD 308 to investigate the degradation of phenyl- and cyclopropyl-^{14}C radiolabelled bifenthrin in two types of sediment : Calwish Abby Lake and Swiss Lake (test material purity: 94%). According to this experiment, bifenthrin was **slowly degraded** in the water/sediment systems, with a $DT_{50\ whole\ system}$ values of 93 d (176 d at 12°C) and 276 d (524 d at 12°C), function of the sediment type (El Naggar, 2003). The mineralization ranged from 3.5% to 27% after 99 days and the maximum amount of bound residues was 14.2% after 99 days. No metabolites ≥ 10% were found in the water phase. 4'-Hydroxy bifenthrin was the only metabolites in sediment ≥ 10% (11.1% on day 99 in the Swiss Lake System)

An other water/sediment study was performed using a pond system and a river system (test material purity: 94%). DT_{50} values were about 320 d for the pond system (608 d at 12°C), and about 180 d in the river system (370 d at 12°C) (Cresswell, 1986)

A case study based on the two water/sediment study is available (Verhaar, 2003). In this study, DT_{50} and K_{om} were recalculated using the TOXSWA compartment model (water, sediment, glass vessel walls). This model indicates a rapid dissipation of bifenthrin from water, especially due to adsorption to sediment or into glass walls. Moreover, the overall dissipation of bifenthrin from the total system is due to degradation in the water phase. In other words, according to the model, almost no degradation takes place in the sediment.

Biodegradation in soils

Several reliable studies, all carried out under GLP, were performed on the rate and route of degradation of cyclopropyl- and phenyl-^{14}C labelled bifenthrin (purity 97.9%) in 4 soil types : silt loam, silty clay loam, sand loam (Smith, 1991; Bixler, 1983 et 1984; Reynolds, 1986). Bifenthrin was slowly degraded in these soils. DT_{50} values normalized to 20 °C were generally > 90 days but < 180 days. The average DT_{50} was 161 d (4 soils, 2 labels). The normalisation at 12°C leads to an average value of 305 days.

4.1.3 Summary and discussion of persistence

According to the studies presented above, biodegradation of bifenthrin is expected to be limited in sediment, water and soil matrices. Bifenthrin is hydrolytically stable in water and photodegradation in natural water is expected to be limited. There was no information or comment during public consultation opposing this conclusion. RAC confirms on this basis that bifenthrin is not rapidly degradable under CLP-criteria.

4.2 Environmental distribution

4.2.1 Adsorption/desorption

A screening adsorption test (according to OECD 106, test material purity of 97.3%) with 4 soils showed that **bifenthrin is very strongly adsorbed to soil**.

Bifenthrin has an average K_{oc} of 236610 $L.kg^{-1}$, range from 130526 to 301611 $L.kg^{-1}$ and therefore is likely to be essentially immobile in the soil. No leaching to groundwater is expected (Froelich, 1984).

4.2.2 Volatilisation

Bifenthrin is not volatile and its vapour pressure is low ($2.431 * 10^{-5}$ Pa at 25°C). It has a moderate volatility from water because its estimated Henry's law constant is 101 Pa m^3 mol^{-1}, which is equivalent with an air-water partition coefficient of 0.04 L/L. However, emission from surface water to atmosphere will not occur because of the strong adsorption of bifenthrin to sediment.

4.2.3 Distribution modelling

No relevant data available.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

Based on its log K_{ow} > 6, bifenthrin is expected to have a high bioconcentration potential. However, BCFWIN v2.15 estimation does not predict a high bioaccumulation factor due to a significant correction related to the cyclopropyl-C(=O)-O- ester. For very hydrophobic products such as bifenthrin, linear equations however are not recommended. Using non-linear model such as Bintein

et al. (1993) where Log BCF = 0.910 log P – 1.975 log (6.8.10-7 P + 1) – 0.786, BCF value of 12589 L.kg^{-1} is estimated using a Log P = 6.6 or 16982 using Log P = 6.

4.3.1.2 Measured bioaccumulation data

Table 2: Summary of bioconcentration studies

Guideline / Test method	Exposure/depuration (days)	Species	Initial concentr. of a.s. [µg/L]	Steady-state whole body BCF [L/kg]	Uptake rate constant	Depuration rate constant	Reference
Method agrees with OECD 305 E	42 / 42 (Flow-through)	*Lepomis macrochirus*	0.0009	6090	Approx. 180 L^{-1}kg^{-1}d^{-1}	0.012 d^{-1}	Surprenant, 1985
OECD 305C	28 (60+28) (Semi-static)	*Lepomis macrochirus*	0.007 0.085	1241 1414	26.92 37.80	0.024 0.032	Gries, 2006
Similar to OECD Guideline 305C	70 d (Flow-through)	*Cyprinus carpio*	0.0085 0.085	666 1082	1.8e-1	3.1e-2	Shigeoka and Saito, 1993
Special higher tier test with sediment in the test system	21 d	*Pimephales promelas* Asellus sp *Daphnia magna* *Corbicula fulminea*	0.24–1.86 0.33 0.24 0.33-2.58	63 146 423 140	ND ND ND ND	ND ND ND ND	Surprenant, 1988
Fish Full Life Cycle Test (US EPA-FIFRA 1458-145, guideline 72-5)	day 127 day 254 newly fertilised 96-h	*Pimephales promelas* F0 F0 Embryos Embryos F1	0.0037, 0.0090, 0.019, 0.040 and 0.090	21000 28000 83 – 4900 530 – 10000 6000	ND ND ND ND	ND ND ND ND	McAllister, 1988

A BCF study was available with common carp (Shigeoka and Saito, 1993). The BCF value for uptake of bifenthrin (purity of test material: 97%) in fish from clean water based on the fitted steady state concentration at the high exposure level is 1082 L.kg^{-1}.(corresponding to 1290 L.kg^{-1} related to total measured radioactivity). The corrected BCF for lipid content of test fish (3.2%) is 2016 L.kg^{-1} (lipid normalized to 5% lipid content)

Two BCF studies were performed with the bluegill sunfish. The purity of the tested substances was not specified. The first one was performed by Surprenant (1985) and lead to BCF values of 6090 L.kg^{-1} (related to total measured radioactivity) based on the ratio of concentration fish/water.

The results of the new study with bluegill sunfish (purity of test material > 95%) performed by Gries (2006) confirm the results of the carp study; indeed the whole steady state BCF was found to be 1414 L.kg^{-1}. The corrected BCF for lipid content of test fish (3.3%) is 2142 L.kg^{-1} (lipid normalized to 5% lipid content).

In addition, in a fish Full Life Cycle Test (US EPA-FIFRA 1458-145, guideline 72-5) conducted with radiolabelled bifenthrin. BCFs (based on the ratio of concentration fish/water) were calculated at several sampling points of the study.

In the parental generation, $BCF_{whole\ fish}$ (*Pimephales promelas*) was 21000 $L.kg^{-1}$ at day 127 and 28000 $L.kg^{-1}$ at day 254 (test material: 10.36% ^{14}C-bifenthrin in hexane with radiopurity of 33.52 mCi/mM). Results from this study however indicate that the steady state was not obtained after 127 days since BCF still increased after 254 days.

On the other hand, experiments carried out in the presence of soil sediment and on test material with a purity of 95% show that the bioconcentration is greatly diminished by the presence of sediment particles with BCF values ranging from 63 to 423, due to preferential adsorption to sediment.

4.3.2 Summary and discussion of bioaccumulation

According to the Guidance to Registration (EC) No 1272/2008 on Classification, Labelling and Packaging of substances and mixtures (part 4), a BCF in fish of $\geq$ 500 $L.kg^{-1}$ is indicative of the potential to bioconcentrate for classification purpose. With several reliable fish bioaccumulation studies available, demonstrating BCFs well above the classification criterion, RAC considers the potential of bifenthrin to bioaccumulate as decisive for environmental classification. There was no information or comment during public consultation opposing this conclusion.

4.4 Secondary poisoning

No data available.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Absorption

Bifenthrin is absorbed via oral route [oral absorption rate of 50% (corresponding to the summation of the urinary and biliary excretion and tissue residues) in rats] (Selim, 1987; El-Naggar and Tullman, 1991) and via dermal route (11.4% absorbed through human skin *in vitro*) (Gelis, 2007). There is no information about the potential of Bifenthrin to be absorbed following inhalation.

Elimination

In a metabolism study in rats, after oral exposure to 4 or 35 mg/kg bw/d, the majority of radioactivity was eliminated via faeces (66-83%) and at a lesser extent in urine (9-25%) within 48-72 hours (Selim, 1987). Biliary excretion was the second most significant excretion pathway (20-30%). No remarkable sex differences in elimination or distribution were observed.

Distribution

Blood bio-kinetics showed that blood level of radioactivity slowly increased with time and reached its peak at 4 and 6 hours after oral administration, following low and high dose administration, respectively, and then slowly declined thereafter (Selim, 1986). In a bioaccumulation study in rats exposed by oral route to 0.5 mg/kg bw/d, the highest levels of residues were detected in fat and skin with parent chemical accounting for the majority of the residue (Hawkins *et al*., 1986). The estimated half-lives were 51 days (fat), 50 days (skin), 19 days (liver), 28 days (kidney), 40 days (ovaries and sciatic nerve). A steady state appeared in plasma concentrations of radioactivity at the 21st day (0.04 to 0.06 µg/ml) and then, decreased rapidly at 78 days and was below <0.01 µg/ml at the remaining sacrifice time. These long biological half-lives were anticipated based on the high log P_{ow} of bifenthrin (log P_{ow} 6.6).

In a range-finding developmental neurotoxicity study in rats (exposed to 0, 50, 65, 80, 100 and 125 ppm in the diet), exposure of the pups to the test article via the milk was determined based on measurements of the test material in milk on lactation days 5, 11 and 17 following dietary administration of the test article from gestation day 6 through lactation day 22 and comparing internal levels in the dams and pups via the blood (Nemec, 2006). The mean levels of bifenthrin in maternal plasma and in milk samples were clearly increased at the highest tested dose level showing that bifenthrin was excreted in breast milk. The pup plasma bifenthrin level was increased at PND4 when the dams were exposed to the highest dose. It could be then assumed that bifenthrin was able to cross the placenta barrier. However, the plasma bifenthrin level was not increased in pups from treated dams at PND22 compared to controls, showing that bifenthrin was not or slightly absorbed from the milk or rate of metabolism was faster in pup rats at PND22.

Metabolism

Bifenthrin metabolism in the rat is similar to other pyrethroids that are also metabolised through typically hydrolysis with formation of the corresponding alcohol, oxidation of the resulting alcohol to the acid followed by a conjugation process (Cheng, 1988; Wu, 1988).

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

Table 3 : Summary of the acute oral toxicity studies

Route	**Method Guideline**	**Purity of test substance**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Clinical signs** Gross abnormalities	**Reference**
Oral	EPA 81-1, OECD 401	93.7% (isomer cis-Z)	Rat, Sprague-Dawley, 5/sex/dose	Males: 100, 150, 200 and 300 mg/kg bw Females: 75, 100, 200 and 300 mg/kg bw (no vehicle used)	LD_{50} combined 186.1 mg/kg bw LD_{50} males: 168.4 mg/kg bw LD_{50} females: 210.4 mg/kg bw	Immediate lethality (day 1) observed at 150, 200 and 300 mg/kg bw. Clinical signs included tremors, vocalisation, clonic convulsions, twitching, abdominal gripping and hypersensitivity to touch. Other signs found were abdominal staining, oral discharge, chromorhinorrhea, chromodacryorrhea, diarrhea and broken tooth. In survivors signs were transient and ended by day 3. No gross internal lesions.	Watt, 1997
Oral	EPA 81-1	91.4% (90% cis/10% trans isomer)	Mice, Swiss Webster, 10/sex/dose	25, 35, 42 and 50 mg/kg bw in corn oil	LD_{50} males: 43.5 mg/kg bw LD_{50} females: 42.5 mg/kg bw	Immediate lethality (day 1) observed in all treated groups. Clinical signs included clonic convulsions, tremors and oral discharge. By day 1 all survivors had returned to normal. No gross internal lesions.	Rand, 1983a

5.2.2 Acute toxicity: inhalation

Table 4 : Summary of the acute inhalation toxicity study

Route	**Method Guideline**	**Purity of the test substance**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Clinical signs** Gross abnormalities	**Reference**
Inhalation	EPA, OPPTS 870-1300; OECD 403	94.8% (96.1% after exposure to the study heating regimen) (isomer cis-Z)	Rat, CrL CR^R (SD)IGS BR, 5/sex/dose	560, 990 and 2300 mg/m^3 (nominal) 4 hours Nose-only (liquid droplet aerosol)	LC_{50} combined 1010 mg/ m^3 LC_{50} males: 1100 mg/m^3 LC_{50} females: 800 mg/m^3	Immediate lethality (day 1) observed in all treated groups (†: 2/10 at 560 mg/m^3, 3/10 at 990 mg/m^3 and 10/10 at 2300 mg/m^3) Clinical signs included abnormal gait, tremors, convulsions, hypothermia, laboured respiration, rales, decreased defecation/urination, increased respiration rate, unkempt appearance and red/yellow staining on various body surfaces. Survivors in the 560 and 990 mg/m^3 groups were normal by day 4 and 10, respectively. Macroscopy revealed red discoloration and/or dark red areas of the lungs and distended gas-filled stomach and sections of the intestines for succumbed animals in all groups. No toxicologically significant effects for survivors in the 560 and 990 mg/m^3 group.	Kiplinger, 2003

5.2.3 Acute toxicity: dermal

Table 5 : Summary of the acute dermal toxicity study

Route	**Method Guideline**	**Purity of the test substance**	**Species Strain Sex no/group**	**dose levels duration of exposure**	**Value LD50/LC50**	**Clinical signs** Gross abnormalities	**Reference**
Dermal	EPA 81-2	88.35% (98% cis isomer)	Rat, Sprague-Dawley, 5/sex/dose	2000 mg/kg bw 24 hours (no vehicle used)	LD_{50} > 2000 mg/kg bw	There were no deaths. Male rats exhibited staggered gait on days 2 and 3. Female rats exhibited staggered gait, decreased locomotion and abdominogenital staining between days 2 and 4. All rats gained weight by termination of the study. No gross internal lesions. No irritation at the test sites.	Kedderis, 1985

5.2.4 Acute toxicity: other routes

No data available in the dossier.

5.2.5 Summary and discussion of acute toxicity

Systemic effects

The acute toxicological profile of bifenthrin is characterised by neurotoxicity (tremors and clonic convulsions). Following acute exposure (by gavage or by inhalation), there is an immediate onset of these transient neurotoxic effects. These neurotoxic effects (if sufficiently pronounced) are considered to be the major cause of immediate lethality. The acute toxicity of bifenthrin was tested in rats and mice: there is no difference in the qualitative toxicological profile of bifenthrin in both species.

Based on the results of the acute oral toxicity studies in rats and mice (LD_{50} rat, male: 168 mg/kg; LD_{50} mouse, female: 42 mg/kg), the dossier submitter proposed to classify bifenthrin with the CLP classification Acute Tox. 3 – H301 and as 'toxic' with the risk phrase **R25 - Toxic if swallowed** according to the Directive 67/548/EEC criteria (corresponding guidance values from 25 to 200 mg/kg).

Considering the comments received in the public consultation the dossier submitter modified its proposal as follows: Acute oral toxicity in mice is more severe than acute oral toxicity in rats. Based on the lowest oral LD_{50} value in mice (42.5 mg/kg in females) the dossier submitter proposed the

CLP classification category Acute Tox. 2 - H300 (CLP guidance values for this category from 5 to 50 mg/kg bw).

Based on the LC_{50} = 800 mg/m^3 in female rats, the dossier submitter proposed the classification category Acute Tox. 3 - H331 based on the CLP criteria and a classification with the risk phrase **R23 - Toxic by inhalation**, according to the Directive 67/548/EEC criteria.

In the acute dermal toxicity study in rats at the tested dose of 2000 mg/kg there were acute clinical effects, but no mortality. Accordingly no acute classification was proposed for the dermal route.

RAC opinion

During RAC discussions it was pointed out, that the CLP classification for acute oral toxicity is supported by the results of the acute toxicity study in mice. Because it was accepted to use these relevant data of the most sensitive species, for acute toxicity RAC confirmed the classification proposals of the dossier submitter as modified after the public consultation.

Local effects (paresthesia)

A literature search was conducted for reports of signs and symptoms of exposure in the general population. Numerous studies reviews of human poisoning with pyrethroids and epidemiological studies have been published. Occupationally, the main adverse effect of dermal exposure of pyrethroids is paresthesia, most commonly affecting the face. Management of pyrethroid toxicity is supportive and symptomatic. As paresthesia usually resolves in 12-24 h, specific treatment is not generally required, although topical application of vitamin E may reduce their severity.

Under Directive 67/548/EEC, the S-phrase S24 should be applied for substances seen to cause paresthesia by skin contact and therefore is proposed for bifenthrin. There is no equivalent precautionary statement under CLP.

5.3 Irritation

5.3.1 Skin

Table 6 : Summary of the skin irritation study

Species	Purity of the test substance	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference
			Erythema	Edema			
New Zealand white Rabbit	88.35 % (98 % cis isomer)	EPA 81-5 (0.5 ml)	0	0	Not applicable	Not irritating	DeProspo, 1983

5.3.2 Eye

Table 7 : Summary of the eye irritation study

Species	Purity of the test substance	Method	Average Score (mean of 24, 48 and 72 h)				Result	Reversi-bility yes/no	Reference
			Cornea	Iris	Redness Conjunctiva	Chemosis			
New Zealand white Rabbit	88.35% (98 % cis isomer)	EPA 81-4 (0.1 ml)	0	0	0.11	0	Not irritating	yes	DeProspo, 1983

5.3.3 Respiratory system

There is no specific information regarding the ability of bifenthrin to cause irritation to the respiratory tract during the acute inhalation toxicity study in rats. It should be additionally taken into account that only very high bifenthrin concentrations have been tested by inhalation, which resulted in mortality in all dose groups tested.

Few human case reports on pyrethrins were reported namely: chest pain, throat irritation, nasal irritation/stuffy nose, respiratory irritation and shortness of breath (Ellenhorn *et al*., 1997). Specific information on e.g. dose-response relationships is not available.

5.3.4 Summary and discussion of irritation

Based on the available data (skin and eye irritation study with rabbits, acute rat inhalation study, few human case reports on pyrethrins) bifenthrin is not considered to be an irritant substance.

RAC opinion

The dossier submitter concluded that a classification for dermal irritation, eye irritation or respiratory tract irritation is not warranted. RAC accepted this proposal of the dossier submitter.

5.4 Sensitisation

5.4.1 Skin

Table 8 : Summary of the sensitisation assay

Species	Method	Purity of the test substance	Number of animals sensitized/total number of animals	Result Concentration used	Reference
Guinea pig	OECD 406, maximization test	94.8% (98 % cis isomer)	15 animals tested (5 control and 10 test) Test group: 8/9* (discrete /patchy erythema) Negative control group: 0/5	Sensitizing Intradermal induction: 5% (in PEG 300). Epidermal induction: undiluted test material Challenge concentration: 3% (in PEG 300)	Arcelin, 2003

** One animal of the test group was found dead on day 18. At necropsy, no macroscopic findings were noted and death was considered to be unrelated to treatment.*

5.4.2 Summary and discussion of sensitisation

Bifenthrin was found to be a skin sensitiser to guinea-pigs in the maximisation test (89% of positive responses at the intradermal induction concentration of 5%).

A classification with **Xi; 'R43: may cause sensitisation by skin contact'** was proposed by the dossier submitter. The classification category **Skin Sens. 1 – H317** was proposed according to CLP.

RAC opinion

No information opposing the proposal was received during the public consultation and RAC discussions. Thus RAC confirmed the proposal to consider bifenthrin as a skin sensitiser as outlined above.

According to the current draft of the 2nd ATP of the CLP regulation strong skin sensitisers are allocated to subcategory 1A, while for the other skin sensitisers with a low or moderate potency the subcategory 1B is foreseen. According to the proposed criteria Bifenthrin is allocated to subcategory 1B (>= 30% responding animals at > 1% intradermal induction dose).

5.5 Repeated dose toxicity

5.5.1 Repeated dose toxicity: oral

Table 9 : Summary of the oral repeated dose and chronic toxicity studies

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral	28 day	91.4% (isomer cis-Z)	Rat (Sprague Dawley), 10/sex/ dose	0, 50, 100, 200, 300 and 400 ppm (equivalent approximately to 0, 4.4, 10.75, 21.9 and 34.5 mg/kg bw/day in males and to 0, 5.4, 11, 21.6 and 32.6 mg/kg bw/day in females)	**400 ppm:** Clonic convulsions and tremors, followed by death of all animals by day 15 of the study No significant treatment-related pathology No treatment related histopathology **300 ppm group:** Clonic convulsions and tremors Mortality: 6/10 males died by day 12 and 1/10 females died by day 20 of the study Significantly elevated adrenal weight and depressed testes weight (males), elevated relative adrenal, brain and kidney weights (males), elevated relative brain, kidney and liver weight (females) No significant treatment-related pathology No treatment related histopathology **200 ppm group:** Tremors in males and in females No significant treatment-related pathology No treatment related histopathology **100 ppm:** No significant treatment-related pathology No treatment related histopathology **50 ppm:** Significantly elevated brain weight (females)	200 ppm (22 mg/kg bw/d)	100 ppm (11mg/kg bw/d)	Rand, 1983b

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
					and significantly elevated brain and kidney weights (males) No significant treatment-related pathology No treatment related histopathology			
Oral	90 day	91.4% (90% cis/10% trans isomer)	Rat (Sprague Dawley), 15/sex/ dose (Recovery group of 10 animals at the highest dose level/control group).	0, 12, 50, 100 and 200 ppm (equivalent approximately to 0, 0.9, 3.4, 7.5 and 15 mg/kg bw/day in males and to 0, 1.05, 4.3, 8.5 and 17.15 mg/kg bw/day in females)	At 100 ppm: tremors in 2/15 males and in 3/10 females At 200 ppm : tremors observed in all the treated animals, subsiding within three days.	100 ppm (7.5 mg/kg bw/d in males and to 8.5 mg/kg bw/d in females)	50 ppm (3.4 mg/kg bw/d in males and to 4.3 mg/kg bw/d in females)	Rand, 1984

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral	90 day	88.35% (isomer cis-Z)	Dogs, Beagle, 4/sex / dose	2.5, 5.0, 10.0, 20.0 mg/kg bw daily by capsule	No mortality Tremors: - At 2.5 mg/kg/d: one ♂ (wk11) - At 5 mg/kg/d: 3/4 ♀ (wk10 to 13) and 3/4 ♂ (wk1, 3, 7, 9, 10, 11, 12, 13) - At 10 and 20 mg/kg/d: all animals displayed tremors throughout the 13-week study Ataxia and languid appearance at 5, 10 and 20 mg/kg bw/d. Pituitary cysts observed at gross necropsy at 20 mg/kg bw/d (4/8) vs 0/8 in the control group. At the histopathological examination, pituitary cysts were also observed in the control group 2/8 vs 3/8 at the highest tested dose.	5.0 mg/kg bw/d	2.5 mg/kg bw/d	Serota, 1984

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral	52-week	88.35% (isomer cis-Z)	Dogs, Beagle, 4/sex / dose	0, 0.75, 1.5, 3.0, 5.0 mg/kg bw daily by capsule	No mortality Delayed tremors in all males and females at 5 mg/kg/day during weeks 15-29 and in one male and 2 females at 3 mg/kg/day during weeks 16-23 with the effect somewhat more pronounced in the males. This effect was first observed following 15 weeks of treatment and disappeared following 29 weeks of treatment. The lack of tremors after 29 weeks suggests that dogs may have developed a tolerance to treatment. In seven of the dogs (2 from the 3 mg/kg/day group and 5 from the 5 mg/kg/day group), tremors were noted prior to the daily dose, indicating a persistent effect from the previous day's dose Decreased body weight gain at 5 mg/kg bw/day. Tendency toward decreased mean erythrocyte count, hemoglobin and hematocrit values at 5 mg/kg bw/d from week 26.	3 mg/kg bw/d	1.5 mg/kg bw/d	Serota, 1985
Oral	2-year (chronic and oncogenicity combined)	88.35% (isomer cis-Z)	Rat, Sprague Dawley 50 /sex/ dose	0, 12, 50, 100 or 200 ppm (equivalent approximately at week 104 to 0, 0.6, 2.3, 4.7 and 9.7 mg/kg bw/day in males and to 0, 0.7, 3, 6.1 and 12.7 mg/kg bw/day in females).	Tremors at 200 ppm and in one female at 100 ppm. Treatment-related decreased body-weight (gain) at 200 ppm in females. Decreased Red Blood Cell levels in males at 200 ppm. No treatment-related effects neither on organ weight, nor at necropsy or at the histopathological examination (including the sciatic nerve examination) .	100 ppm (4.7 mg/kg bw/day for males and 6.1 mg/kg bw/day for females	50 ppm (2.3 mg/kg bw/d for males and 3 mg/kg bw/d for females)	McCarty, 1986

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Oral (food)	2-year	88.35% (isomer cis-Z)	Mice, Swiss Webster, 50/sex/ dose	0, 50, 200, 500, 600 ppm ad libitum in diet (equivalent approximately at termination to 0, 7.6, 29, 74 and 92 mg/kg bw/day in males and to 0, 10, 37, 93 and 110 mg/kg bw/day in females.	Lethality at 500 ppm (1♀/50) and at 600 ppm (2♂/50 and 2♀/50). Minimal clinical signs of toxicity observed in males at 200 ppm (tremors). From 500 ppm (♂ and ♀) mainly, clinical signs of toxicity such as tremors, jerks, twitching and convulsions, occurring during the first three months of the study. Although in-life clinical observations identify the nervous system as the target system, there was no evidence of damage to these tissues at the microscopic level From 500 ppm (♂ and ♀), decrease of food consumption and body weight gain. Statistically significant increase in retinal atrophy at 600 ppm (♂♀). Statistically significant increase in bilateral germinal epithelial degeneration in testes from 50 ppm, without any dose-response relationship. The etiology of this change is obscure even though the incidence figures indicate an association with treatment.. No evidence of damage of the nervous system at microscopic examination	200 ppm (29 mg/kg bw/d for males) and 500 ppm (93 mg/kg bw/d for females), based on tremors..	50 ppm (7.6 mg/kg bw/d for males) and 200 ppm (37 mg/kg bw/d in females).	Geiger, 1986

5.5.2 Repeated dose toxicity: inhalation

No data available.

5.5.3 Repeated dose toxicity: dermal

Table 10 : Summary of dermal repeated dose study.

Route	duration of study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Results	LOAEL	NOAEL	Reference
Dermal	21 day	93.2% (isomer cis-Z)	Rat (Sprague Dawley), 10/sex/ dose	0, 25, 50, 100, 1000 mg/kg bw/day (exposure duration: 6 hours)	Tremors observed in 9 females at 1000 mg/kg bw/day. Staggered gait observed in 1 male and in 1 female at 100 mg/kg bw/day (day 1 and 2) and in 1 female at 1000 mg/kg bw/day (day 1and 2), exaggerated hindlimb flexion observed in 1 female at 100 mg/kg bw/day and in 4 females at 1000 mg/kg bw/day (day 2 and 4). Local irritation observed from day 7 of observation and from 25 mg/kg/d. Paraesthetic reaction (vocalization, thrashing in cage and lying on back) observed during the first half-period of treatment (until day 11 at the maximum) at - 25 mg/kg/day and 50 mg/kg bw/day: one female - 100 mg/kgbw/day: one male and one female - 1000 mg/kgbw/day: one male and six females Hyperplasia with increased severity at 1000 mg/kg bw/day marked in males, moderate to marked in females), sometimes associated with ulceration and secondary dermal inflammation.	25 mg/kg bw/d (local)	50 mg/kg bw/d (systemic)	Watt *et al*., 2000

5.5.4 Summary and discussion of repeated dose toxicity:

Chapter 5.5.4 consists of two parts: the original summary and discussion of the dossier submitter and a second part referring to RAC discussions and the RAC proposal.

Original summary of data of the dossier submitter

Oral application

Bifenthrin has been tested for chronic oral toxicity in dogs, rats and mice. In these studies, the most sensitive treatment-related toxic effect observed was the occurrence of tremors. Tremors and convulsions are considered to be serious adverse effects; at dosages with marked tremors and convulsions lethality does occur as well (e.g. 28-day study in rats).

Signs of neurotoxicity (tremors and convulsions) were observed either at the beginning of the study (in mid and high-dose groups) or as delayed effects throughout the exposure period (in low-dose groups). In some of the repeated-dose studies (rats, mice, dogs) it is described that tremors induced at specific dosages subsided with duration of exposure and did not persist up to the end of the study. For the rat, a broad spectrum of durations of exposure has been tested: to some extent there is a decrease of LOAELs for tremors and corresponding lethality with increasing duration of exposure.

Consistently no histological damage of the nervous system was observed. In general, pyrethroids could exert neurotoxic effects by disturbing nerve impulses (in particular, via their action onto the voltage-dependant sodium channel of excitatory nerves, to alter permeability of the sodium ion) (Miyamoto *et al*., 1995).

Dermal application

In a 21-day dermal rat study, systemic toxicity was observed at the dose range between 100 and 1000 mg/kg/d (staggered gait and exaggerated hindlimb flexion at the beginning of the study, tremors). The clinical signs “vocalization, trashing in cage, and lying on back” were considered indications of pyrethroid-induced paresthesia (see acute dermal toxicity as to the labelling of bifenthrin with S24).

Classification proposal

Original classification proposal of the dossier submitter

According to the findings of the 90-day rat study, tremors are observed from 100 ppm (approx. 8 mg/kg bw/d). Based on the Directive 67/548/EEC criteria, a classification with Xn; R48/22 is justified when serious damages are observed between 5 and 50 mg/kg/d by oral route in 90-day studies and Xn; R48/22 is therefore proposed for bifenthrin. Besides, a classification with STOT Rep.1 – H372 is also considered, according to the CLP criteria (threshold for classification in cat. 1 ≤10 mg/kg/d). Based on tremors, it is proposed to identify the nervous system as the primary target organ by oral and dermal routes. No data are available by respiratory route and it is therefore proposed to allocate to the hazard statement H372 the following additional statement for target organ but not for route of exposure: H372 (nervous system).

RAC discussions

Some comments received during public consultation supported the general line of justification of the dossier submitter; other comments questioned the proposed classification. The difference in opinions is mainly related to the issue whether to consider the clinical signs of neurotoxicity (tremors and convulsions) in the chronic studies as repeated dose toxicity or as acute toxicity. RAC discussed this issue in detail:

The following table relates to (1) the dependence of LOAELs for clinical signs of neurotoxicity to duration of exposure and (2) to the relationship between dose levels for clinical signs of neurotoxicity and lethality. Reference is made to both the original CLH dossier and the DAR (draft assessment report).

Table: Bifenthrin LOAELs for clinical signs of neurotoxicity and lethality

		Acute Toxicity	28-day study	90-day study	2- year feeding study (rat, mouse) 1- year gavage study (dog)
Rats	Clinical signs of neuro-toxicity	20 or 34 mg/kg (LOAEL) NOAELnot available Tremors declined within few days 20/34 mg/kg was the lowest dose tested Most critical data based on 3 acute oral rat studies (DAR)	22 mg/kg/d (LOAEL) 11 mg/kg/d (NOAEL) No detailed description of time course (DAR)	7.5 mg/kg/d (LOAEL) 3.4 mg/kg/d (NOAEL) Tremors subsided only within the three days of initiation of the post-treatment period showing a clear recovery (DAR).	4.7 mg/kg/d (LOAEL) 2.3 mg/kg/d (NOAEL) Only rudimentary description of the time course of symptoms. However: the incidence of tremors decreased during the middle portion of the study and increased later towards the termination of the study (DAR)
	Lethality:	40 mg/kg (LOAEL) 20 mg/kg (NOAEL)	33 mg/kg/d (LOAEL) 22 mg/kg/d (NOAEL)	No lethality at highest dose of 15 mg/kg/d	No lethality at highest dose of 9.7 mg/kg/d

Mice	Clinical signs of neuro-toxicity	25 mg/kg (LOAEL) NOAEL not available By day 1 all survivors had returned to normal			29 mg/kg/d (LOAEL) 7.6 mg/kg/d (NOAEL) Clinical signs during the first 3 months of the feeding study; clinical signs subsequently disappeared (DAR)
	Lethality:	Lethality at 25 mg/kg/ 25 mg/kg was the lowest dose tested			Lethality at 74 mg/kg/d
Dogs	Clinical signs of neuro-toxicity			5 mg/kg/d (LOAEL) 2.5 mg/kg/d (NOAEL) Definite increase in the incidence of tremors as the study continued (DAR)	3 mg/kg/d (LOAEL) 1.5 mg/kg/d (NOAEL) "Tremors observed following 15 weeks of treatment and disappeared following 29 weeks of treatment" (DAR)
	Lethality:			No lethality at highest dose of 20 mg/kg/d	No lethality at highest dose of 5 mg/kg/d

LOAELs resp. NOAELs for clinical signs of neurotoxicity indicate that there is an impact of the duration of exposure on these values; however, this impact is rather small and can only be recognised for the rat data (for mice acute and chronic LOAELs for tremors seem to be similar, for dogs acute toxicity data are not described). As far as data allow for, a small increase of those dose levels revealing clinical signs of neurotoxicity results in lethality as well. In some studies the quotient between the LOAEL for lethality and clinical signs of neurotoxicity is not more than a factor of 2; in some other studies this factor cannot be calculated but seems to be a little bit higher.

The information on the time-dependent course of the clinical signs of neurotoxicity at specific dose levels is rather limited and seems to depend critically on the dose level chosen (whether the specific dose level results in rather small or serious clinical effects). In the 2-year rat feeding study the

incidence of tremors decreased during the middle part of the study and then increased again towards the end of the study. In the 2-year mice feeding study clinical signs of neurotoxicity were transient and disappeared during the course of the study. For the 90-day dog gavage study the incidence of tremors are reported to increase with duration of exposure; while for the 1-year dog gavage study clinical signs disappeared towards the end of the study. Thus the chronic manifestation of neurodysfunction critically seems to depend on specific finally unknown conditions of the experimental design of the corresponding studies.

The following table contains a comparison of the effective doses for clinical signs of neurotoxicity with the study type -specific guidance levels for RDT classification. The guidance levels chosen for the different durations of exposure and for the different experimental animal species are those that have been pragmatically used in recent RAC documents. The current rule of RAC is that for a specified duration of exposure there are identical guidance levels for different species. Overall, this comparison indicates effective doses for clinical signs of neurotoxicity fulfilling the STOT RE 1 criteria, but generally not fulfilling the DSD criteria for the corresponding category of R48/25.

Table: Guidance levels for RDT classification and effective bifenthrin doses (in mg/kg/d)

Species	Duration of exposure	R 48/22	R 48/25	STOT RE 2	STOT RE 1	Non-effec-tive dose	Effective dose (tremors and con-vulsions)	Resulting classi-fication (CLP criteria)
Rat	28 days	150	15	300	30	11	**22**	STOT RE 1
Rat	90 days	50	5	100	10	3.4	**7.5**	STOT RE 1
Rat	2 years	6.25	0.625	12.5	1.25	2.3	**4.7**	STOT RE 2
Mice	2 years	6.25	0.625	12.5	1.25	7.6	**29**	-
Dog	90 days	50	5	100	10	2.5	**5**	STOT RE 1
Dog	1 year	12.5	1.25	25	2.5	1.5	**3**	STOT RE 2

RAC recognised that bifenthrin did not result in pathology or histopathology of the nervous system; the critical effects to be discussed are clinical signs of neurotoxicity (mainly tremors and convulsions). The CLP regulation explicitly covers significant/severe reversible effects for RDT classification: "Target organ toxicity (repeated exposure) means specific, target organ toxicity arising from a repeated exposure to a substance or mixture. All significant health effects that can impair function, both reversible and irreversible, immediate and/or delayed are included" (chapter 3.9.1.1 of CLP regulation). Thus it is the opinion of RAC that a RDT classification is adequate for reversible clinical signs of neurofunctional disorders even if no irreversible histomorphological damage to the nervous tissues has been demonstrated.

The central question is whether these adverse effects finally should be classified as acute or repeated dose toxicity. The current guidance on the application of the CLP criteria comments on this issue: "Where the same target organ toxicity of similar severity is observed after single and repeated exposure to a similar dose, it may be concluded that the toxicity is essentially an acute (i.e. single exposure) effect with no accumulation or exacerbation of the toxicity with repeated exposure. In such a case classification with STOT-SE only would be appropriate" (commentary to CLP Annex I 3.9.1.6). Thus the relevant question is whether the clinical signs of neurotoxicity in acute and repeated dose testing are of similar severity at similar doses. Based on the available data on all species tested, it is difficult to recognise differing degrees of severity. For the purpose of this proposal for classification, it is assumed that the LOAELs for clinical neurotoxicity are indicators of similar severity. With this definition the general conclusion is, that target organ toxicity of similar severity following repeated dose is observed at a somewhat lower dose than following a single exposure (see second last table). However, the difference in effective doses is small; with the consequence of a controversial discussion of the need for repeated dose toxicity classification.

There have been statements in favour of not classifying for repeated dose toxicity: The adverse effect in question (tremors and convulsions) in principle is considered to be an acute effect because one effective dose leading to an effective plasma concentration is sufficient to elicit this type of effect. Tremors and convulsions are the critical adverse effects in the acute studies. Bifenthrin is a Type 1 pyrethroid. The common mode of action of this group of substances ("sodium channels") is recognised as an acute mode of action. These acute symptoms of intoxication are considered to be covered by the classification for acute toxicity (Acute Tox. 2 – H300) because the difference in the dose levels for marked clinical signs of neurotoxicity and lethality is small. The message from the classification for acute toxicity (Acute Tox. 2 – H300) is that even single exposure in experimental animals resulted in lethality (combined with tremors and convulsions) at a dose range of 5 to 50 mg/kg/d.

There were other contributions to the discussions stressing a different perspective: the mode of action was not considered to be an essential criterion; the observed clinical signs of neurotoxicity at the LOAELs reported were evaluated significant and severe, irrespective of the observation that in some studies these adverse effects declined with duration of dosing. The doses which elicited these functional adverse effects in acute and repeated dose testing were considered to be sufficiently different to justify an additional classification for repeated dose toxicity. With reference to the rat data, there is experimental evidence, that the acute LOAEL for the clinical signs of neurotoxicity of about 20 mg/kg (or somewhat lower) decreases to a 2-year LOAEL of about 5 mg/kg/d.

RAC opinion

RAC finally concluded to give special weight to the descriptive dose-response data indicating that target organ toxicity (clinical signs of neurotoxicity) for repeated exposure is observed at lower dosages than for single exposure. For relevant studies, the effective doses for the clinical signs of neurotoxicity were lower than the lower CLP guidance levels thus resulting in a classification with STOT RE 1. Because of different DSD guidance values, the less severe category R48 / 22 is warranted. With this opinion RAC follows the initial recommendation of the dossier submitter.

5.6 Mutagenicity

5.6.1 In vitro data

Table 11: Summary of the *in vitro* mutagenicity studies

Test system Method Guideline	**organism/ strain(s)**	**Purity of the test substance**	**concentra-tions tested**	**Result**		**Remark**	**Reference**
				+ S9	**- S9**		
Ames test (comparable to EEC B.14)	*Salmonella typhimurium:* strains TA1535, TA1537, TA98, TA100, TA1538	91.4% (isomer cis-Z)	0, 75, 375, 1875, 3750, 7500 µg/plate	-	-	No cytotoxicity. Test compounds precipitated at 333 µg/plate and upwards. Positive-control compounds yielded expected responses. No replication of the experiment	Haworth, 1983
Mammalian chromosome aberration test - 92/69/EEC Method B10	Chinese hamster Ovary (CHO) cells	35 % (isomer cis-Z)	1, 2.5, 5, 10 mg/mL	-	-	Test concentrations were based on preliminary cytotoxicity testing. Positive-control compounds yielded expected responses. No replication of the experiment or confirmation of the result with different time of exposure, very short time of exposure with metabolic activation (2 hours), 50 cells analysed per culture instead of 100 as recommended by technical guideline.	Thilagar, 1984a
HGPRT assay - 87/302/EEC Method B17	CHO cells	88.3% (isomer cis-Z)	20 - 100 µg/ml	?	?	Equivocal It is not known if the equivocal results are observed with or without S9. Cytotoxic doses not reported	Thilagar, 1984b

Test system Method Guideline	organism/ strain(s)	Purity of the test substance	concentra-tions tested	Result		Remark	Reference
				+ S9	- S9		
Unscheduled DNA synthesis - 87/302/EEC Method B18	Primary rat hepatocytes	Data not available in the study report	0.01 - 2.0 µg/ml	-	No data	Equivocal in a first assay Negative in the replicate Cytotoxic doses not reported	Thilagar, 1983a Thilagar, 1983b
Sister chromatid exchange assay in mammalian cells - OECD 479	Chinese hamster Ovary (CHO) cells	88.35% (isomer cis-Z)	1, 5, 10, 30, 60, 100 µL	-	-	Test concentrations were based on preliminary cytotoxicity testing. Positive-control compounds yielded expected responses.	Heidemann , 1989
Mammalian cell gene mutation test - 87/302/EEC Method B17	Mouse lymphoma L5178Y cells (TK +/-)	88.3% (isomer cis-Z)	Without S9 mix: 0.013-1 µL/mL With S9 mix: 0.0013-0.1 µL/mL	+	+	The test was carried out with 1/8 log dilutions of the concentrations giving rise to 100% toxicity This gene mutation test (TK) with tissue culture cells showed a positive response. The dossier contains, , another type of gene mutation test (HGPRT), with CHO cells and one with mouse lymphoma cells. One of these was inconclusive due to a positive effect in at a lower concentration, while the two other yielded negative results. Positive-control compounds yielded expected responses.	Putman, 1983a

5.6.2 In vivo data

Table 12 : Summary of the *in vivo* mutagenicity studies

Type of test Method/ Guideline	Species Strain Sex no/group	Purity of the test substance	frequency of application	sampling times	dose levels	Results	Remarks	Reference
Cytogenetics assay - 92/69/EEC Method B11	Rat, Sprague Dawley , 5 males/ group	91.1% (isomer cis-Z)	1 per day for 5 consecutive days	4-8 h after the fifth daily treatment.	3, 10 and 30 mg/kg bw/ day	No apparent change in ploidy. No effect on mitotic index. Incidence of aberrations and number of aberrations per cell not statistically significantly increased in treated groups.	Negative and positive control compounds yielded expected responses for determination of valid test. 50 metaphases analysed per animals instead of 100 as recommended by	Putman, 1983b

Type of test Method/ Guideline	Species Strain Sex no/group	Purity of the test substance	frequency of application	sampling times	dose levels	Results	Remarks	Reference
							technical guideline.	
Micronucleus test-OECD 474	Mice, ICR, 5 animals/sex/group	94.7%	Single oral administration (gavage)	24 and 48 hours	8.75, 17.5, 35 mg/kg	No significant increase in the incidence of micronucleated PCEs in mouse bone marrow was observed at dose up to 35 mg/kg (at 24 hours) or at 35 mg/kg (48 hours post-dosing). Clinical signs of toxicity were observed from 8.75 mg/kg but no modification of the ratio P/N was observed.	The maximum dose was determined in a range-finding study where lethality was observed from the lowest tested dose, namely 50 mg/kg. Therefore, 35 mg/kg was chosen as the maximum dose level.	Krsmanovic and Hudson, 2005
Unscheduled DNA Synthesis test – OECD 486	Rat-Sprague Dawley	94.7%	Single oral administration (gavage)	2- 4 hours and 12-16 hours	0, 7.5, 15 and 30 mg/kg	No significant increase in the mean number of net nuclear grain counts in hepatocytes isolated either 2 to 4 hours or 12 to 16 hours after dose administration up to the highest tested dose (30 mg/kg).	The maximum dose was determined in a range finding study where tremors were observed from 40 mg/kg and lethality from 75 mg/kg. No individual data reported, only mean reported. Poor response of the positive control group at 2-4 hours with value lower than the historical values.	Kamala Pant and Sly, 2005

5.6.3 Human data

No data available

5.6.4 Other relevant information

No data available

5.6.5 Summary and discussion of mutagenicity

Bifenthrin yielded negative results *in vitro* in the Ames test (Haworth, 1983), in the chromosome aberration assay in CHO cells (Thilagar, 1984a), and in a SCE in CHO cells (Heidemann, 1989). Positive results were observed in a gene mutation assay on mouse lymphoma L5178 Y cells with detection of trifluorothymidine resistance (Putman, 1983a). Bifenthrin showed equivocal results in another gene mutation assay (HPRT) in CHO cells (Thilagar, 1984b) and in an *in vitro* unscheduled DNA synthesis (UDS) assay (Thilagar, 1983a), but the replicate yielded negative responses (Thilagar, 1983b). However, the three available *in vivo* genotoxicity assays were negative: an *in vivo* chromosome aberration assay in rats (Putman, 1983b), a mouse micronucleus assay (Krsmanovic and Hudson, 2005) and a rat UDS assay (Kamala Pant and Sly, 2005).

RAC opinion

Based on these available mutagenicity data, the dossier submitter did not propose a classification for mutagenicity.

No information opposing this evaluation was received during the public consultation and RAC discussion. Thus, specifically based on the negative findings in all the in vivo genotoxicity assays, it was confirmed by RAC not to propose a classification for germ cell mutagenicity.

5.7 Carcinogenicity

This chapter on carcinogenicity consists of the original non-revised version of the dossier submitter followed by a section that contains additional data and a summary of relevant RAC discussions.

5.7.1 Carcinogenicity: oral

Table 13 : Summary of carcinogenicity data *(Original table of the dossier submitter)*

Route	Duration of treatment/study	Purity of the test substance	Species Strain Sex no/group	dose levels frequency of application	Tumours	Reference

Oral (food)	104 weeks	88.35% (isomer cis-Z)	Rat, male Sprague Dawley, female Tac (SD) fBR, 50/sex/dose	0, 12, 50, 100, 200 ppm *ad libitum* in diet	No treatment-related tumour induction up to 200 ppm NOAEL (systemic) = 50 ppm (tremors)	McCarty, 1986
Oral (food)	104 weeks	88.35% (isomer cis-Z)	Mice, Swiss Webster, 50/sex/dose	0, 50, 200, 500, 600 ppm *ad libitum* in diet (corresponding in males to 0, 7.6, 29, 74 and 92 mg/kg bw/d and in females to 0, 10, 37, 93 and 110 mg/kg bw/d respectively).	No statistically significant compound-related effects on survival. Increased incidence of pericytoma (initially qualified as leiomyosarcoma) in the urinary bladder in males from 50 ppm (corresponding to 7.6 mg/kg bw/d) statistically significant at 600 ppm (92 mg/kg bw/d). The incidence was as follows: 2/48 (4%), 6/50 (12%), 8/50 (16%), 7/50 (14%) and 14/49** (29%) in males at 0, 50, 200, 500, 600 ppm respectively. Statistically significant increased incidence of lymphoblastic lymphosarcoma and leukaemia at 600 ppm in females. The incidence for lymphoblastic leukaemia was as follows: 12/50 (24%), 14/50 (28%), 17/50 (34%), 10/50 (20%) and 22/49* (44%) in females at 0, 50, 200, 500, 600 ppm respectively. Slight dose-related increased incidence of liver adenocarcinoma and adenoma in males from 200 ppm but not statistically significant. The incidence for combined tumours was as follows: 2/49 (4%), 2/50 (4%), 4/50 (8%), 4/50 (8%), 7/49 (14%) in males at 0, 50, 200, 500, 600 ppm respectively. Slight increased incidence of bronchiolar-alveolar adenocarcinoma and adenoma, statistically significant at 50, 200 and 600 ppm, without dose-related relationship. The incidence was as follows: 14/50 (28%), 26/50* (52%), 23/50* (46%), 19/50 (38%), 23/48* (48%) at 0, 50, 200, 500, 600 ppm	Geiger, 1986

					respectively in females. NOAEL (systemic) = 50 ppm (♂)-200 ppm (♀) ppm (tremors) NOAEL (tumours) ≤ 500 ppm	

5.7.2 Carcinogenicity: inhalation

No data available.

5.7.3 Carcinogenicity: dermal

No data available.

5.7.4 Carcinogenicity: human data

No data available.

5.7.5 Other relevant information

No data available.

5.7.6 Summary and discussion of carcinogenicity

Original study summaries of the dossier submitter

Rat study

The oncogenicity study in Sprague Dawley rats (McCarty, 1986) indicated that bifenthrin is not oncogenic. In this study, tremors were observed at 100 and 200 ppm in females and at 200 ppm in males. The NOAEL for systemic toxicity was set at 50 ppm, based on tremors.

Mice study

In the oncogenicity study in Swiss Webster mice (Geiger, 1986) increased incidence of leiomyosarcoma in the urinary bladder were observed in males at 50, 200, 500 and 600 ppm (statistically significant at 600 ppm). These tumours were slowly growing and did not metastasize. After re-evaluation of this study by a panel of pathologists, it was concluded that the mouse bladder tumour was not a leiomyosarcoma but rather a tumour arising in the submucosa. This latter tumour has an unknown pathogenesis, may arise from the vascular mesenchyme and may be qualified as a pericytoma (predominantly benign).

Other tumours such as lymphoblastic lymphosarcoma and leukaemia were observed in females and are statistically significant at the very high dose (600 ppm).

Besides, statistically significant bronchiolar-alveolar adenocarcinoma and adenoma were observed in females at low, medium ad very high dose.

Original classification proposal of the dossier submitter

"Based on the available information, it cannot be considered that these effects are not relevant to human as long as mechanistic explanations or further information are not provided showing that these tumours are specific to the mice and cannot be extrapolated to human.

Overall, bifenthrin presents:

- No carcinogenic effect in rats
- A carcinogenic effect in mice
- An absence of genotoxic effect or other supporting evidence for carcinogenicity
-

Based on induction of tumours in one species without supporting evidence, a classification **Carc. Cat. 3; R40** is proposed. Because evidence of carcinogenicity in mice is obtained from a single study, it is considered that there is a "limited evidence of carcinogenicity effects" which deserves a **classification Category 2 – H350** according to CLP criteria."

Public consultation and RAC discussions

The dossier submitter proposed to classify bifenthrin for carcinogenicity (CLP Carc. Cat 2). The comments received during public consultation indicated that there is additional information relevant for the assessment of bifenthrin carcinogenicity. Industry submitted this additional information. The various issues raised have been discussed by RAC and are summarised in the following paragraphs. The main discussions relate to the adequacy of the study duration and the top dose level of the mice carcinogenicity study, the adequacy of statistical decision criteria for tumour types with relatively high control incidences, and the relevance of the empirical evidence of increased tumour rates in the liver and urinary bladder of male mice.

Carcinogenicity: Study length, survival and MTD (male Swiss Webster mice)

There was a comment questioning the validity of the mouse carcinogenicity study because a 24-month duration of the study was considered too long. With reference to the Draft Assessment Report (2006) RAC noticed that the duration of the mouse carcinogenicity study was shorter than 24 months; the duration of the study was shortened in order to maintain a sufficient general survival of experimental animals. The duration of treatment was shortened to 78 weeks; the overall duration of the study was 89 weeks for males and 91 weeks for females.

In the relevant testing guidelines there is indeed a discussion on the optimal study length for different strains of mice. Depending on the specific strain of mice used, a study length between 18 and 24 months is recommended. The main idea is that at the end of the study there should be a sufficient survival of experimental animals in the control and low dose groups. There is the general recommendation that the number of survivors in these experimental groups should not be lower than about 25%. The following table indicates that the survivals in the mice study with bifenthrin clearly fulfill this condition of the 25%-rule. Thus it is the opinion of RAC that the mice study design sufficiently followed the EU and OECD testing guideline recommendations as to the optimal duration of dosing. Thus findings at the top dose level cannot be simply dismissed because of the study length chosen.

Table: Survival of male and female Swiss Webster mice in the bifenthrin study

	Controls	50 ppm	200 ppm	500 ppm	600 ppm
Male survival in % (week 78)	48	56	68	44	68
Male survival in % (end of study)	28	38	48	26	38
Female survival in % (end of study)	36	26	30	42	36

During public consultation the issue was raised not to account for the high dose findings in the mice carcinogenicity study because the MTD (maximum tolerated dose) was considered to be exceeded.

Clinical signs of toxicity (predominantly dose-related tremors) were noted at the two highest dose levels. These findings were reversible: they occurred during the first tree months of the study and subsequently disappeared.

2 males of the high dose group died after 1 to 2 weeks of the study possibly as a result of compound-related acute toxicity. However, chronic exposure to bifenthrin even at the highest dose had no influence on longevity. Male survival at week 78 (end of treatment) and at the end of the study at the highest dose was higher than in the control animals.

With reference to the original study report (Geiger 1986) the following dose-dependent retardations in body weight gains were calculated:

Table: Body weight gains in male and female Swiss Webster mice

Retardation in body weight gain	Control	50 ppm	200 ppm	500 ppm	600 ppm

in %					
Male mice (week 27)	-	-7.1	-4.3	-9.9	-14.9
Male mice (week 78; end of treatment)	-	-16.6	-11.4	-8.6	-9.1
Male mice (end of study)	-	-18.8	-19.9	-11.4	-13.6
Female mice (week 27)	-	-6.9	-4.6	-8.5	-2.3
Female mice (end of study)	-	-5.0	-6.1	-15.0	-9.4

The concept of the maximum tolerated dose (MTD) for carcinogenicity studies generally is to select a top dose that should ideally provide some signs of toxicity such as a slight depression of body weight gain (but not more than 10% relative to controls) without substantially altering normal life span due to effects other than tumours. RAC considers this 10% value as important point of orientation, but not as a strict demarcation line.

With reference to the table above the retardation in body weight gain is more pronounced in male mice than in female mice. In male mice the retardation in body weight gain at the top dose level is higher than the proposed reference value of 10%. In the first weeks of the study there are marked clinical signs of toxicity and a relative high retardation in body weight gain at the top dose level. However, during the further course of the study clinical signs of toxicity disappeared and the reduction in body weight gain did not show a clear dose-response relationship anymore. In the late phase of the study (e.g. week 78 and at the end of the study) the highest retardation of body weight gain is at the lowest doses. Thus, at least in terms of body weight gain and survival, chronic exposure to bifenthrin at both top dose levels does not seem to have weakened the animals' health status. It is recommended in the draft OECD guidance No. 116 that for compounds that are not genotoxic the top dose should be informed by considerations of MOA; for bifenthrin specific MOA data are not available. RAC concludes that it has not been shown that the elevated tumour incidences at the highest dose level are linked to an unspecific weakening of the health status of the exposed animals. Thus RAC recognises no sufficiently convincing limitation of the study design in order to dismiss the findings at the highest dose level. Furthermore, CLP classification criteria do not require not to classify for carcinogenicity if the MTD is exceeded, but leave the decision for a carcinogenicity category 2 still open.

<u>Carcinogenicity. Statistical decision criteria</u>

During public consultation it was proposed to use the "rule of Haseman" to statistically assess increases in tumour incidences. Haseman (1990) recommended a significance level of $P < 0.01$ for common tumours and of $P < 0.05$ for rare tumours. The definition of a rare tumour is an incidence of less than 1%, based on historical controls. At spontaneous incidences above 1% tumours are considered common. This procedure has been proposed to control for false positive tumour rates (to reach a close agreement between statistical significance and decisions on biological significance). However, current EU/OECD testing guidelines do not specify such a rule (e.g. OECD testing guideline 451). In the OECD draft guidance document 116 the appropriate selection of a specific significance level is discussed without advising a specific decision rule. It is stressed that the selection of a statistical decision rule is a policy choice based on a trade-off between the risks of false positive and false negative tumour rates. RAC recognises the rationale for a differentiated statistical decision rule for rare and common tumours. So far, RAC prefers to stick to the conventional 5% decision rule; however RAC recognises that such a statistical decision rule is more a general guidance than a strict demarcation line for solving the question whether the adverse effects observed should be considered treatment-related.

Trend tests and pairwise comparison tests are the recommended tests for determining whether chance rather than a treatment-related effect is a plausible explanation for an apparent increase in tumour incidence. Significance in either kind of test is sufficient to reject the hypothesis that chance accounts for the results. This approach is proposed in the OECD draft guidance document No. 116; this recommendation is referred to because it seems that in some of the comments to the CLH report a treatment-related effect is rejected in case of a non-significant pairwise comparison test even if there is statistical significance in a trend test.

Carcinogenicity: re-evaluation of histopathological slides in mice study

Following corresponding industry comments during public consultation the Rapporteur requested a robust study summary of the report on the re-evaluation of the original sections from the mouse bifenthrin carcinogenicity study. The robust study summary and the corresponding original report were submitted by industry and has been considered by RAC.

The re-evaluation of the histological slides referred to urinary bladders of all males and females, and to liver sections of all male mice and lung sections of all female mice. All slides were reviewed in a blind evaluation by the first reviewer (this is the information from the robust study summary; the original report itself only expresses that "bladders from all male and female mice have been reviewed by Butler"). Only the slides with bladder lesions were reviewed by two further pathologists. Statistical analysis of the urinary bladder findings was based on the majority opionion.

To facilitate RAC decision finding a summary and discussion of the relevant tumour findings (original evaluation and re-evaluation) is presented in the following:

Carcinogenicity: lung tumours in female Swiss Webster mice

Table: Lung tumours in female Swiss Webster mice (tumour incidences in %)

Tumour type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Reference
Bronchio-alveolar adenomas and carcinomas	28	52* p=0.012	46* p=0.048	38	48* p=0.041	Geiger 1986 (cited from CLH dossier)
Adenomas	24	44* p=0.029	38	30	40	Butler 1991 (Original and RSS)
Carcinomas	4	8	8	8	4	Butler 1991 (Original and RSS)
Bronchio-alveolar adenomas and carcinomas	28	52* p=0.013	46* p=0.049	38	44	Butler 1991 (Original and RSS)

There is no essential difference in both histopathological assessments of lung tumours available. The only difference refers to the incidences in the 600 ppm group (48% versus 44 % in the re-evaluation). The incidence of bronchio-alveolar adenomas and carcinomas was increased compared to concurrent controls (P values between 0.01 and 0.05). There was already a relatively high incidence in the controls (28%). In all test groups, there were elevated tumour incidences of about 40 to 50%; without any dose-response relationship. The range of historical controls is reported to be between 4% and 57% (RSS of Butler 1991; no further information on the adequacy of historical data). It is the conclusion both of the study pathologist and the reviewer, that this incidence pattern of lung tumours should not be considered compound-related (DAR 2006, Butler 1991). RAC as well does not recognise sufficient evidence for a causative role of bifenthrin for the increased incidences of lung tumours.

Carcinogenicity: lymphoid tumours in female Swiss Webster mice

Table: Lymphoid tumours in female Swiss Webster mice (tumour incidences in %)

Tumour type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Reference

Lymphoblastic leukemia	24	28	34	20	44* p=0.024	Geiger 1986 (cited from CLH dossier)
Lymphoid tumours (including lymphoblastic leukemia)	38	38	40	32	47	DAR 2006

For lymphoid tumours there was no histological re-evaluation of tissues. For lymphoblastic leukaemia, a large number of control animals was affected (24%). The incidence in high dose females was statistically significant (P value between 0.01 and 0.05). The trend test does not show statistical significance, the dose response is not monotonic (lowest incidence at 500 ppm).

When combining all types of lymphoid tumours (including lymphoblastic leukaemia) there was no statistical significance in pairwise comparisons (combining of these types of lymphoid tumours is considered common practice). A large number of control animals was affected (38%). The dose response is not monotonic (again a decline of incidence at 500 ppm below the control incidence). There is no information on historical controls. It was the conclusion of the study pathologist that the observed incidence pattern was not compound-related. RAC as well does not consider the lymphoid tumours as treatment-related.

Carcinogenicity: liver tumours in male Swiss Webster mice

Table: Liver tumours in male Swiss Webster mice (tumour incidences in %)

Tumour type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Reference
Adenomas	4	4	6	4	10	Geiger 1986 (cited from DAR)
Adenocarcinomas	0	0	2	4	4	Geiger 1986 (cited from DAR)
Adenomas and adenocarcinomas	4	4	8	8	14 trend p=0.022	Geiger 1986 (cited from DAR)
Adenomas	2	2	0	4	6	Butler 1991 (Original and RSS)

Adenocarcinomas	0	0	2	4	4 trend p=0.024	Butler 1991 (Original and RSS)
Adenomas and adenocarcinomas	2	2	2	8	10	Butler 1991 (Original and RSS)

In the original evaluation there is a positive trend test for combined liver adenomas and adenocarcinomas; in the re-evaluation the only significant result reported is a positive trend test for adenocarcinomas. Pairwise comparisons did not reveal significance. It was the conclusion of the study pathologist (DAR 2006) and of the experts of the re-evaluation that the liver tumours were unlikely to have been treatment-related (Butler 1991). The main arguments for rejecting a treatment-related effect have been the assumption of a relatively high historical control incidence for these liver tumours and the non-significance in pairwise comparison tests.

For CD-1 mice historical control incidences of 0-16% for adenomas and 6-28% for adeno-carcinomas are reported (no further information on average values, number of animals and studies and on the time window of retrospective analysis of studies; no further references). These historical control data cannot be considered sufficiently valid. There is one further relevant study with Swiss Webster mice that was conducted at the same laboratory during approximately the same time period as the bifenthrin carcinogenicity study (as reported by Gammon et al., 2011). In this study male control mice had a 2% incidence of liver adenomas. For liver adenocarcinomas there was a 0 % incidence in the controls and the three lowest doses. At the highest dose level there was a 2 % incidence for these liver adenocarcinomas.

Concurrent control incidences are rather low (no adenocarcinomas, 2% or 4% adenomas, depending on the pathologist). The only relevant additional study available clearly supports the weight and relevance of the zero incidence for liver adenocarcinomas in the concurrent control group. Thus there is no valid evidence that these liver tumours are to be considered as common tumours in this strain of mice. In combination with the positive trend tests and the rather similar incidences of adenocarcinomas and combined adenomas and adenocarcinomas at the two highest (very similar) dose levels it is the interpretation by RAC that the hypothesis that chance accounts for the results in liver can be rejected; RAC thus assumes a treatment-related weak carcinogenic effect of bifenthrin in the liver of male mice.

Even if there would have been a treatment-related carcinogenic effect in the liver of male mice industry proposed (FMC 2011) to consider the bifenthrin liver tumour findings as not relevant for humans. With reference to experience with other pyrethroids, industry assumes a phenobarbital mode of action for these liver tumours. However, because in the male mice bifenthrin study there are no non-neoplastic findings in the liver and there are no bifenthrin-related MOA investigations RAC is not in the position to judge the relevance of this proposed mode of action and to account for these considerations for classification purposes. Based on the data available, RAC recognises a weak treatment-related dose response for bifenthrin liver carcinogenicity.

Carcinogenicity: urinary bladder tumours in male mice

The following table contains the original data together with the reevaluated urinary bladder tumour data. There are two relevant changes: (1) the urinary bladder tumours are reclassified (from malignant leiomyosarcomas to benign submucosal bladder tumours, (2) the re-evaluation resulted in a marked increase of the corresponding control incidence data.

Table: Tumours in the urinary bladder in male Swiss Webster mice (tumour incidences in %)

Tumour type	Control	50 ppm	200 ppm	500 ppm	600 ppm	Reference
Leiomyosarcomas	4	12	16	14	29** p<0.01 _____ trend positive	Geiger 1986 (cited from CLH dossier)
Submucosal mesenchymal urinary bladder tumours:	12	14	16	16	27 p=0.068 _____ Trend positive with p=0.046	Butler 1991 (RSS) Butler et al., 1997

Submucosal mesenchymal urinary bladder tumours including early lesions:	14	14	18	16	30 p=0.05 _____ Trend positive with p=0.033	

Morphology of urinary bladder tumours in male mice

In the re-evaluation by Butler (1991) the tumours originally described as leiomyosarcomas were re-diagnosed as submucosal mesenchymal tumours. The review pathologists considered these submucosal bladder lesions as benign tumours without any evidence of metastases.

In 1997 the California EPA (Cal/EPA) completed a human health risk assessment on bifenthrin. For the assessment of carcinogenicity the re-evaluation of Butler (1991) had been taken into account. Cal/EPA concluded that the urinary bladder tumours should be classified as urinary bladder sarcoma-NOS. Cal/EPA stated that their concern for tumours remained because of a higher ratio of invasive tumours and masses in the higher dose groups.

RAC recognises that there has been a discussion in the literature on the degree of malignancy of these urinary tract tumours. There are statements finally indicating that these lesions might not be tumours at all (Karbe 1999).

Cohen (2011) expressed the view that the overall interpretation of the mesenchymal lesions is that they present benign proliferations in the mouse urinary bladder. The tumours are described to occur predominantly in the submucosa occasionally extending into the muscle layer. According to Cohen, this does not actually represent muscle invasion, as it does not destroy the muscle layers themselves. “Whether these lesions actually represent benign neoplasms or whether they represent an aberrant inflammatory and granulation tissue response continues to be debated, although the evidence increasingly suggests that it is an inflammatory, reactive disorder” (Cohen 2011).

RAC recognises the ongoing discussions and diagnostic uncertainties on the morphology and degree of malignancy of the urinary bladder lesions. With reference to the morphological description of the urinary bladder tumours by Butler (1991) RAC is of the opinion that these lesions are to be considered as tumours. RAC accepts the approach to consider these tumours as benign tumours. However, there are structural elements which are characteristic for a transition from a benign to a malignant tumour (such as pleomorphy of cells and nuclei and invasion into

surrounding tissues). In order to justify this consideration the morphology of these lesions observed is described in some more detail:

> In the re-evaluation (Butler 1991, Butler et al., 1997) selected urinary bladder sections were stained with PTAH1. Electron microscopy of five tumours initially reported as leiomyo-sarcomas showed evidence of myofilaments indicative for smooth muscle in epitheloid and spindle cells. The lesions originally described as leiomyosarcomas were re-diagnosed as benign submucosal bladder tumours without any evidence of metastases. The tumours were usually single but in some instances in two distinct areas. A few tumours protruded into the lumen of the bladder and occasionally became polyploid. Tumours showed both epitheloid and spindle cells, which formed irregular and abnormal vascular channels with red blood cells. In many areas spindle cells had oval nuclei and had the form of smooth muscle. Invasion of the spindle cell component into and through the muscle wall was present in some cases. Mitoses were sparse but were observed in many tumours. In other areas of the tumours, epitheloid cells predominated and appeared as large bizarre shaped (pleomorphic) cells with large hyperchromatic nuclei and basophilic and eosinophilic inclusions. Chronic inflammatory infiltrate around the edge, areas of necrosis, and hemosiderin were common observations in submucosal tumours. Where possible, the reviewers located tumours in the trigone region of the urinary bladder. The histogenesis could not accurately be defined but was considered to derive from vascular mesenchyme rather than from the smooth muscle of the bladder wall.
>
> In addition to the lesions considered to be tumours a lesser number of smaller, poorly circumscribed submucosal lesions were also observed that showed the same spindle cell morphology and vessels of the tumours but did not contain foci of epithelioid cells. These lesions were assumed to be early stages of tumour development.

Historical control data

In the re-evaluation by Butler (1991) it was stressed that there are no reliable data on historical control incidences of these submucosal mesenchymal tumours. As major reason methodological difficulties in correctly diagnosing this tumour type was stated. Butler (1997) argued that in the 1950s a variety of diagnostic terms have been employed to record this lesion. With this degree of diversity in nomenclature the compiling of reliable historical control data would require a review of the examined urinary bladders in order to confirm the diagnosis.

Such an effort was undertaken by the International Life Science Institute (ILSI). In a review on 17 carcinogenicity studies (15 on CD-1 mice, 2 on Swiss mice) containing approximately 8000 mice ILSI found an overall incidence of 1.2 % with a range of 0-17% in the combined set of control and treated males (Halliwell 1998). In 15 studies incidences were at 2% or below, for only two studies higher tumour incidences (6.8% and 17%) were observed. RAC recognised that the highest incidence in the publication by Halliwell (1998) with high probability is this bifenthrin case. Since also treated animals were included in the ILSI review no spontaneous incidences specifically for

[1] PTAH phosphotunstic acid hematoxylin to demonstrate striated muscle fibers

control animals were identified. In case of treatment-related increases of tumour incidences in these studies the actual control incidences for urinary bladder tumours would be lower than reported.

No submucosal mesenchymal tumour was observed in the benalaxyl carcinogenicity study in 60 control Swiss male mice.

In an addendum to the carcinogenicity study on sulfosulfuron (California EPA 2005) it was stated that historical control data (assumed to refer to CD-1 mice from the Monsanto database) from 16 studies on benign mesenchymal urinary bladder tumours showed incidences of 1/910 for males and 0/931 for females.

Halliwell (1998) discusses that these submucosal bladder tumours might be underreported. It was suggested that the incidence was probably higher than published since many submucosal urinary bladder tumours are very small, only being recognised on histopathology and the common tissue trimming procedure of cross-sectioning the bladder does not provide adequate examination of the trigone area where these tumours were assumed to be located most often. It was stated that these tumours were more likely observed if the bladder is sectioned midsagitally than in those bladders cut cross-sectional. However, in Halliwell (1998) unfortunately there was no differentiation of the reviewed oncogenicity studies as to this obviously important tissue trimming procedures.

With respect to historical control incidences there is one additional relevant study with Swiss Webster mice that was conducted at the same laboratory during approximately the same time period as the bifenthrin carcinogenicity study (as reported by Gammon et al., 2011). In male mice the reported tumour incidences for “leiomyosarcomas” of the urinary bladder are: 8% in controls (4/49), 11% at dose 1 ((3/28), 6% at dose 2 (2/35), 15% at dose 3 (4/26) and 10% at dose 4 (5/49).

Overall, it is the opinion of RAC that the empirical evidence available does not prove that there is a high spontaneous rate for these submucosal mesenchymal urinary bladder tumours in Swiss and CD-1 male mice.

Dose response of urinary bladder tumours in male mice

The re-evaluation of the urinary bladder tissue slides resulted in a change in tumour incidences. A significant increase of tumour incidences was reported in the control group (from 4% in the original report to 12% in the re-evaluation); the tumour incidences in the treated groups remained similar. In the original evaluation there was a positive trend with a significant increase at the top dose level ($p<0.01$). The results of the re-evaluation were of borderline statistical significance (trend test with $p=0.046$ and pair-wise comparison with $p=0.068$ at the top dose level).

Cal/EPA did not consider the peer-review process in the re-reading of slides sufficient to support a revision of the tumour incidences because the overall tumour incidences were not reviewed by all three pathologists. This was considered to be an important issue especially in the situation that the incidence in the controls was raised substantially while the incidence of all other treatment groups remained similar to the original readings.

With reference to the discussion of historical control data it is considered evident that at least the high dose incidence of the urinary bladder tumours (nearly reaching 30%) is far out the range of historical controls. Recognising a positive trend in both evaluations, not dismissing the clear statistical significance of the original evaluation for the top dose level, RAC concludes that sufficient evidence for a treatment-related effect of bifenthrin in the urinary bladders of male mice is available.

Mode of action and human relevance

Available mutagenicity data indicate that the bifenthrin-related tumours are not caused by a genotoxic mode of action.

A severe chronic inflammation of the bladder wall, which was more severe in male mice than in females was reported to be a consistent nonneoplastic finding. Butler et al. (1997) assumed tumours as a manifestation of chronic inflammatory and repair processes due to the observation that chronic inflammatory cell infiltration and hemosiderin were often associated to tumours. However, no details on incidences and severity grades of submucosal inflammatory infiltration and no data on whether they were located at perivascular sites or more diffusely are available. Depending on the tumour type inflammatory cells are commonly observed in and around tumour tissue. Also hemosiderin can often be seen in areas of necrosis in tumours and is commonly seen in tumours with vascular origin. Based on the data available it is the opinion of RAC that the assumption of an inflammatory process as mode of action is not finally substantiated. Furthermore, available data do not allow for a clear description of the specific pathogenesis (Halliwell 1998). Overall it is the opinion of RAC that available data do not allow to describe a specific mode of action for these bifenthrin-related urinary bladder tumours in male mice.

Industry suggested that the mesenchymal urinary bladder tumours should be considered as unique to Swiss and CD-1 mice. It is emphasised (e.g. Cohen 2011) that this specific type of urinary bladder tumours has not been reported in other species including humans. RAC acknowledges this empirical evidence, but wants to stress that because of the methodological problems in correctly diagnosing these lesions, there still might be unknown cases of this or similar urinary bladder lesions in other strains of mice, or other animal species and humans: RAC recognises that a specific analysis of non-urothelial tumours in other mouse strains is not included in this evaluation. No final recommendation on adequate diagnostic terms of submucosal bladder tumours is given. This tumour type is not expected to be reported as a ‘submucosal bladder tumour’ since the international harmonised classifications on tumours in humans or rodents (such as WHO) don’t use the site as diagnostic term for a tumour. RAC does not exclude that this tumour type has not yet been diagnosed in humans because exposure to substances with the hazard of inducing this type of urinary tract tumours has been rather low.

RAC recognises that there are several types of non-urothelial tumours reported for man, rat and mouse. It is known that non-urothelial neoplasms are rare in humans and account for less than 5% of urinary bladder tumours (Dahm and Gschwend 2003). In this review, in a total of 192 reported cases of adult bladder sarcoma, leiomyosarcomas are the most common type of sarcoma. There is similarity among species that non-urothelial tumours are rare in man and mice. In the opinion of RAC it cannot be excluded with certainty that a counterpart of the male mice urinary bladder lesions may exist in man (although expected to be diagnosed more accurately towards its prevalent histomorphologic type). RAC recognises the diagnostic difficulties to unequivocally characterise the non-urothelial tumours.

The central question to RAC is whether the current information that a lesion similar to the mouse mesenchymal proliferative lesion has not been reported in humans is clearly indicative that it does not occur in humans (as proposed by Cohen 2011) or that it cannot be induced in humans.

According to Cal/EPA the weight of evidence of a positive bioassay outcome could only be lessened if a type of tumour occurs exclusively in animals through a demonstrated mechanism known to be irrelevant to humans. Because there were no mechanistic data and no definition of the histogenesis of the tumours, according to Cal/EPA there were no convincing arguments that the tumours found in mice were not relevant to humans.

RAC similarly is of the opinion that not having observed this specific type of tumour in humans does not necessarily mean that this or similar types of tumours cannot be induced in humans. RAC does not presume that necessarily the identical type of tumour is to be induced in bladder tissues of humans or other species; instead the male mice urinary tract tumour data are taken as indication of a carcinogenic potential of bifenthrin that possibly might be expressed in a way that is different to the expression in male mice. Site concordance between experimental animals and humans have not been consistently demonstrated for many substances. RAC concludes that the available evidence does not exclude the human relevance of the male mice urinary bladder tumours.

<u>RAC opinion on carcinogenicity of bifenthrin</u>

Bifenthrin did not result in increased tumour incidences in male and female rats. Bifenthrin is not considered to be an *in vivo* mutagen. However, increased tumour incidences have been reported for male and female Swiss Webster mice which require discussion and assessment.

In female mice increased incidences of lung and lymphoid tumours have been observed. For both types of tumours concurrent control incidences are rather high (in the range of 30% to 40%). For both tumour types the incidence data do not indicate a clear dose-response relationship. RAC does not assume that the increased incidences of lung and lymphoid tumours have been induced by bifenthrin. Both for the lung and lymphoid tumours in female mice RAC concludes that the available evidence does not give sufficient evidence to support a classification for carcinogenicity.

In male mice increased incidences of liver and urinary bladder tumours are reported. RAC considers the experimental design of the male mice carcinogenicity study adequate and acceptable. Survival of control and dosed experimental animals did not fall below the proposed guidance value of 25%. While there have been acute adverse effects and a retardation in body weight gain exceeding the

10% value in the first weeks of the study, chronic exposure to bifenthrin finally did not significantly affect body weight gain and survival. RAC concludes that it has not been shown that the elevated tumour incidences at the highest dose level are linked to an unspecific weakening of the health status of the exposed animals.

There is a weak increase in the incidence of liver tumours (adenomas and adenocarcinomas) in male mice which is considered treatment-related. There was a dose-dependent trend in the development of the adenocarcinomas; the relevance of the concurrent control incidence of 0% is not questioned because there are no convincing data indicating a spontaneous character of these specific tumours. With reference to discussions on pyrethroids it has been proposed to assume a phenobarbital-like mode of action for these liver tumours; this consideration is not taken into account by RAC because of missing bifenthrin-related MOA data.

The increased incidence of the urinary bladder tumours in male mice is considered treatment-related as well. It is the opinion of RAC that the high dose incidence of nearly 30% cannot be explained by a spontaneous occurrence of these tumours. This type of urinary bladder tumours have not been observed in other experimental species and humans. It is the opinion of RAC that this information cannot be used to dismiss the human relevance of the male mice urinary bladder tumour data.

Thus, RAC concludes that there is sufficient evidence to assess the increased tumour rates in the liver and the urinary bladder of male mice as treatment-related. The experimental data indicate that the carcinogenic potential of bifenthrin is weak and has only been expressed in one species and one sex. Available data do not convincingly indicate that these tumours might not be relevant for humans.

RAC concludes that these bifenthrin carcinogenicity data do not fulfill the criteria for the CLP carcinogenicity 1B category. The remaining question is whether the data available are sufficiently positive for a CLP Cat. 2 category or, respectively, sufficiently negative for not classifying bifenthrin for carcinogenicity. The CLP regulation broadly specifies the criteria that trigger a non-classification: negative findings, excessive doses, a high spontaneous tumour incidence, no equivalent tissues or effects not considered relevant for humans because of a specific mode of action or an overly susceptability in a tested species compared to humans. RAC does not consider the high dose level in the male mice carcinogenicity study as excessive. For both types of tumours (liver, urinary bladder), there are no reliable data that describe a high spontaneous tumour incidence or a specific mode of action in male mice. Thus the relevance of these tumours for humans cannot be excluded.

Based on the weak, but clearly recognisable carcinogenic potential of bifenthrin in the liver and urinary bladder of male mice, comparing these data with the relevant classification criteria, RAC concludes to propose a CLP cat. 2 classification for bifenthrin. Carc. Cat. 3, R40 is proposed according to the Directive 67/548/EEC criteria. With this opinion RAC follows the initial recommendation of the dossier submitter.

Addendum: Benalaxyl study

Submucosal mesenchymal bladder tumours in mice and their implications for classification had been addressed by the European Chemicals Bureau (ECB) in the review of the plant protection product benalaxyl (Portugal Ministry of Agriculture 2001). Industry specifically referred to this review when commenting on the relevance of these tumours for classification of bifenthrin. In the Swiss mice oncogenicity study on benalaxyl there was no dose-related increase in tumour incidences in males and females except for 3 urinary bladder tumours in males at the highest dose level tested (3/60). Based on the original study pathologist's diagnosis (transitional cell carcinoma in the urinary bladders) originally category 3 for carcinogenicity was proposed for benalaxyl. In that context a pathology working group considered the original diagnosis as incorrect and considered all three lesions to be submucosal mesenchymal tumours as described by Halliwell (1998). RAC recognises that these urinary bladder lesions may be identical to the urinary bladder lesions in the bifenthrin study; thus in principle the EU discussion on these benalaxyl lesions is considered relevant for the assessment of bifenthrin carcinogenicity as well.

In short: the morphology of these submucosal urinary bladder tumours was considered to be well established, the lesion was considered unique to mice (Swiss Webster and CD-1), its counterpart has not been reported in any other laboratory species or in humans. Its non-epithelial nature was considered to be important since the vast majority of spontaneous and chemically induced mouse and human urinary tumours are of epithelial (= urothelial/transitional cell) origin. Data on historical control incidences were referenced; it was stated that for different reasons the true spontaneous incidence is not known. It was conceded that there still was a controversy as to the aetiology, pathogenesis and biology of the lesions including whether or not the urinary bladder lesion should be classified as a tumour. Based on the overall data available the Commission Working Group on the Classification and Labelling of Dangerous Substances decided not to classify benalaxyl for carcinogenicity (ECBI/62/02 Rev.3).

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR/WHO) concluded that these tumours can occur spontaneously at a high incidence (about 12% in this strain) and did not consider them to be treatment-related. It was stressed that this kind of lesion is non-epithelial in origin, unique to the mouse urinary bladder and has no counterpart in any other species, including humans. JMPR/WHO concluded that there was no evidence of carcinogenic potential of benalaxyl (Vleminckx and Dellarco 2005).

RAC is aware of the Commission's decision not to classify the plant protection product benalaxyl for carcinogenicity. In the Swiss mice oncogenicity study on benalaxyl there was no dose-related increase in tumour incidences in males and females except for 3 urinary bladder tumours in males at the highest dose level tested (3/60). The result of this benalaxyl study is clearly different to the result of the bifenthrin study with a nearly 30% incidence of urinary bladder tumours at the top dose level. Already because of this significant difference in dose response it is evident that the carcinogenicity classification for benalaxyl and bifenthrin need not necessarily be identical.

5.8 Toxicity for reproduction

5.8.1 Teratogenicity

Table 14 : Summary of developmental toxicity data

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Purity of the test substance	Exposure Period	Doses	Critical effects dams fetuses	NOAEL maternal toxicity	NOAEL Teratogenicity Embryotoxicity	Reference
Oral (gavage)	EPA 83-3	Rabbit, New Zealand White, 20/sex/ dose	88.35% (isomer cis-Z)	Day 7-19 post mating	2.67, 4.0, 8.0 mg/kg/day (corn oil)	Tremors and twitching in dams. No major malformations in foetuses were noted. Foetotoxicity was suspected based on abortions and early delivery observed at mid and high doses. The most of the animals showed clinical signs attributed to an infection to *Pasteurella multocida* so results of abortion and early delivery were not considered as relevant.	2.7 mg/kg bw/day	= 2.7 mg/kg bw/day	DeProspo, 1984
Oral (gavage)	EPA 83-3	Rat , Sprague Dawley, 25 females/ dose	88.35% (isomer cis-Z)	Day 6-15 post mating	0, 0.5, 1, 2 mg/kg b w/day (corn oil)	Intermittent tremors (between day 10 & 19) in dams. There were no major malformations noted in any of the fetuses from groups 1 through 4. Minor malformations were observed sporadically and were not considered to be related to test material administration.	1 mg/kg bwday	≥2 mg/kg bw/day	DeProspo, 1984b

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Purity of the test substance**	**Exposure Period**	**Doses**	**Critical effects dams fetuses**	**NOAEL maternal toxicity**	**NOAEL Teratogenicity Embryotoxicity**	**Reference**
Oral (food)	EPA OPPTS 870.3700	Rat, Sprague Dawley, 25 females/ dose	95.3% (isomer cis-Z)	Day 6-20 post mating	30, 60, 90, 200 ppm in diet (2.5, 5, 7.4, 16.3 mg/kg bw/day), respectively)	Clinical signs of neurotoxicity at 200 ppm, decrease of food consumption at 200 ppm, body weight gain and adjusted (for gravide uterine weight) body weight gains in dams at 200 ppm. No treatment- related changes were observed in number of live and dead fetuses, fetal weights, or sex ratios.	90 ppm (equivalent to 7.4 mg/kg b.w./ day)	≥200 ppm (equivalent to 16.3 mg/kg b.w./day)	Watt *et al*., 2001

5.8.2 Fertility

Table 15 : Summary of fertility toxicity data

Route of exposure	Test type Method Guideline	Species Strain Sex no/group	Purity of the test substance	Exposure Period	Doses	critical effect	NOAEL Parental		NOAEL F1		NOAEL F2		Reference
							male	female	male	female	male	female	
Oral (food)	EPA 83-4	Rat, Tac (SD) fBR, 25 animals/sex/ dose	88.35% (isomer cis-Z)	From start of study until sacrifice of parent, F1, F2-generation	30, 60, 100 ppm ad libitum equivalent to 1.5, 3 and 5 mg/kg b.w./day)	Tremors in females of parent and F1 generation There were no treatment-related effects on reproductive parameters (mating, male fertility, female fertility and gestation indices)	S&R ≥ 100 ppm (5 mg/kg b.w./day)	S = 60 ppm (3 mg/kg b.w.) R≥100 ppm (5 mg/kg b.w.)	S&R ≥ 100 ppm (5 mg/kg b.w.)	S= 60 ppm (3 mg/kg b.w.) R≥100 ppm (5 mg/kg b.w.)	S&R >≥ 100 ppm (5 mg/kg b.w.)	S= 100 ppm R≥100 ppm (5 mg/kg b.w.)	DeProspo, 1986

5.8.3 Summary and discussion of reproductive toxicity

Bifenthrin was evaluated for the embryo/foetotoxicity and teratogenicity potentials by oral route in rabbits and rats.

No evidence of teratogenicity or embryotoxicity up to maternally toxic doses was observed after diet or gavage administration of bifenthrin. However, foetotoxicity was suspected in rabbits based on abortions and early delivery observed at mid and high doses. Nevertheless, as most of the animals showed clinical signs attributed to an infection to *Pasteurella multocida*, results of abortion and early delivery were not considered as relevant, possibly due to *Pasteurella multocida*.

The multi-generation reproduction study in rats showed no evidence of fertility toxicity. A slightly but significant decrease of ovary weights was observed in the F_1 generation but not in the F_2 generations. Moreover, a statistically lower live birth index and a statistically higher incidence of stillborn pups were observed solely in the F_{2a} litter and were not dose-related.

Based on the available data, the dossier submitter concluded that bifenthrin is not to be considered a reproductive toxicant and therefore is not to be classified for fertility impairment or developmental toxicity.

RAC opinion

No information opposing this evaluation and proposal was received during the public consultation and RAC discussion. Thus, based on the data available it was confirmed by RAC not to propose a classification for reproductive toxicity.

5.9 Neurotoxicity

5.9.1 Neurotoxicity

Table 16 : Summary of neurotoxicity data

Route	Duration of study	Purity of the test substance	Species Strain Sex no/group	Dose levels frequency of application	Results	LOAEL	NOAEL	Reference

Oral (gavage)	43 days	88.3% (isomer cis-Z)	Domestic laying hen, hybrid brown laying strain, 10 females/ dose	5000 mg/kg bw, single doses at day 0 and 21.	Clinical signs of neurotoxicity at 5000 mg/kg but no delayed neurotoxicity effects observed.	5000 mg/kg bw (neurotoxicity)	5000 mg/kg bw (delayed neurotoxicity)	Roberts *et al*., 1984
Oral (diet)	91 days	93.7% (isomer cis-Z)	Rat Sprague Dawley	0, 50, 100 and 200 ppm	Clinical signs of neurotoxicity (tremors, twitching, FOB) from 100 ppm No microscopic lesions of the nervous system tissues at the highest tested dose level.	100 ppm (6 and 7.2 mg/kg bw/d in males and females respectively)	50 ppm (2.9 and 3.7 mg/kg bw/d in males and females respectively)	Freeman, 1988

5.9.2 Developmental neurotoxicity

Table 17 : Summary of neurotoxicity data

Route of exposure	**Test type Method Guideline**	**Species Strain Sex no/group**	**Purity of the test substance**	**Exposure Period**	**Doses**	**Critical effect**	**NOAEL**				**Reference**
							Maternal neurotoxicity	**Maternal systemic and reproductive toxicity**	**Developmental toxicity (body weights, clinical findings, mortality)**	**Developmental neurotoxicity**	
Oral (diet)	Range-finding study	Rat	94.8%	From gestation day 6 through lactation day 22	0, 50, 65, 80, 100 and 125 ppm (3.6, 4.6, 6.0, 7.4 and 9.3 mg/kg bw/day during gestation and 9.2, 11.7, 14.3, 17.2 and 22.5 mg/kg bw/day during lactation	Tremors and clonic convulsion at 125 ppm	100 ppm (in females)	-	-	-	Nemec, 2006
Oral (diet)	OECD 426	Rat	94.8%	From gestation day 6 through lactation day 21	50, 100 and 125 ppm (3.6, 7.2 and 9 mg/kg bw/d during gestation and 8.3, 16.2 and 20.7 mg/kg bw/day during lactation)	Clinical signs of neurotoxicity from 100 ppm in dams (mainly during lactational period) and at 125 ppm in offspring. Changes in auditory startle and motor activity from 100 ppm in offspring.	50 ppm	≥ 125 ppm	≥ 125 ppm	50 ppm	Nemec, 2006

5.9.3 Summary and discussion of neurotoxicity study

In an acute oral delayed neurotoxicity study in adult hens, bifenthrin did produce signs of neurotoxicity (unsteadiness, jerking movements of the head, trembling, violent movements of the head and legs and inability to stand), but did not show signs of delayed neurotoxicity or histopathological lesions of the nerve tissue.

In a range finding developmental neurotoxicity study, bifenthrin was administered in the diet during gestation and lactation. The only significant effect observed in parental animals was whole-body tremors. No effects were observed on neonatal survival.

In the developmental neurotoxicity study, exposure-related overt signs of maternal neurotoxicity (tremors, clonic convulsions) were observed during gestation and lactation. In offspring, no significant effects were observed on survival, post natal growth body weight. No test article-related macroscopic findings were noted. Clinical signs of neurotoxicity were observed at similar doses in dams and offspring.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

In a standard study (Spruit W.E.T et al., 2002), bifenthrin was found not to exhibit any explosive properties.

No classification for explosivity is proposed.

6.2 Flammability

In standard studies (Spruit W.E.T et al., 2002) bifenthrin was found to be none highly flammable, it did not exhibit any pyrophoric properties and it has no self-ignition temperature.

No classification for flammability is proposed.

6.3 Oxidising properties

Examination of the chemical structure of bifenthrin establishes that it does not contain any chemical groups typical for oxidizing agents. Thus the active substance can be regarded as incapable of reacting exothermically with a combustible material such as powdered cellulose.

No classification for oxidising properties is proposed.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

7.1.1.1 Fish

Short-term toxicity to fish

Several acceptable studies are available and all performed under GLP. Four studies were conducted with *Onchorynchus mykiss* or *Lepomis macrochirus*, following relevant standard test (EPA, 1975) using a flow through test design. 96-h LC_{50} values ranged from 0.1 µg.L^{-1} to 0.35 µg.L^{-1}.

Two additional higher tests with sediment are available. Both tests are conducted with *Onchorynchus mykiss*. The toxicity of bifenthrin was tested in static water/sediment system under repeated spray conditions (two applications, interval 4 days). The results were expressed as initial concentrations deduced from the measured stock solution concentrations. Recovery of the test substance ranged from 30.0 to 58.8% at the test initiation and it was not possible to determine the concentrations for some test conditions after 4 days (before the second application) or 8 days, due to dissipation of the substance from the water column. In this case the 96-h LC_{50} was 0.00626 mg.L^{-1}.

Table 18: Summary of the acute toxicity to fish

Guideline / Test method	Species	Endpoint / Type of test	Exposure		Results			Relia-bility	Reference
			design	duration	LC_0 (µg/L)	LC_{50} (µg/L)	LC_{100} (µg/L)		
EPA, 1975	*Oncorynchus mykiss* *(purity of test material not known)*	Mortality	Flow through	96 h	<0.094[1]	0.15[1]	0.38[1]	2	LeBlanc (1983c)
EPA, 1975	*Lepomis macrochirus* *(purity of test material not known)*	Mortality	Flow through	96 h	<0.180[1]	0.35[1]	0.42[1]	2	LeBlanc (1983b)
EPA, 1975	*Lepomis macrochirus* *(purity of test material not known)*	Mortality	Flow through	96 h	0.1[2]	0.26[2]	0.4[2]	1	Surprenant (1985a)
EPA, 1975	***Oncorynchus mykiss*** (test material: 10.36% 14C-Bifenthrin in hexane with radiopurity of 33.52 mCi/mM)	**Mortality**	**Flow through**	**96 h**	**0.03**[2]	**0.1**[2,4]	**0.3**[2]	**1**	**Surprenant (1985c)**

Higher tier test with sediment	*Oncorynchus mykiss* *(purity of test material not known)*	Mortality	Static	96 h	1.3[3]	6.26[3]	>9.7[3]	2	Aufderheide (1999a)
Higher tier test with sediment	*Oncorynchus mykiss* *(purity of test material not known)*	Mortality	Static	96 h	9[3]	>9[3]	-	2	Aufderheide (1999b)

[1] Based on nominal concentrations (no measures of test concentrations were carried out)
[2] Based on mean-measured concentrations
[3] Based on the concentrations measured in the stock solutions (measured nominal concentrations)
[4] Mean of measurements ranging 0.086 – 0.12µg/l

Conclusion: It is proposed to retain as key study for the classification, the work of Surprenant (1985c): **LC_{50} = 0.1 µg a.s.L^{-1}** (mean measured concentration). Therefore, due to the EC_{50} value lower than 1 mg/l, it is consistent with classification as R50 or H400.

Long-term toxicity to fish

Two studies are available for chronic toxicity to fish. The first study is a flow-through ELS test (larval survival) using the freshwater fish *Oncorhynchus mykiss*. This study was performed following OECD 210 guideline on 10.36% ^{14}C-bifenthrin in hexane with radiopurity of 33.52 mCi/mM. The 76-d NOEC was 0.012 µg.L^{-1} (mean measured). In this study the survival of fertilised eggs in controls and solvent control were very low (19-29%). With a view to the validity criteria of OECD 210 (> 66% hatching success, > 70% post-hatch success), this study cannot be considered as fully reliable.

In a second study, *Pimephales promelas* was exposed to bifenthrin in a flow through full life cycle test design during 120d. This study was performed following EPA 72.5 guideline. The purity of the test material was not specified. The 120-d NOEC was 0.04 µg.L^{-1}.

Conclusion: The chronic toxicity of bifenthrin to fish is very high.

7.1.1.2 Aquatic invertebrates

Short-term toxicity to aquatic invertebrates

Several acceptable studies, all performed under GLP were performed with *Daphnia magna*, *Cerodaphnia dubia*, *Thamnocephales platyurus*, a caddisfly species, and *Gammarus pulex*, following relevant standard test guidelines (ECC C.2, EPA OPP 72-2) and using flow through or static test design. 96-h LC_{50} values ranged from 0.11 µg.L^{-1} to 5.7 µg.L^{-1}.

An additional higher test with sediment is available. An EC_{50} value of 2.3 µg.L^{-1} is observed with *Daphnia magna*.

Table 19: Summary of the acute toxicity to invertebrates

Guideline / Test method	Species/ Endpoint	Exposure		Results			Relia-bility	Reference
		design	duration	EC_0 [µg/L]	EC_{50} [µg/L]	EC_{100} [µg/L]		

OECD 202 EEC C.2.	*Daphnia magna*/ Mortality *(purity of test material not known)*	Flow-through	48 h	<0.60[1]	1.6[1]	>10[1]	2	LeBlanc (1983a)
EPA OPP 72-2	***Daphnia magna*/ Mortality** (test material: 95% ^{14}C-Bifenthrin with radiopurity of 33.52 mCi/mM)	**Flow-through**	**48 h**	**<0.025[2]**	**0.11[2]**	**>0.48[2]**	**1**	**Surprenant (1985b)**
Higher tier test with sediment	*Daphnia magna*/ Mortality *(purity of test material not known)*	Static	48 h	0.49[3]	2.3[3]	10.3[3]	2	Aufderheide (1999)
OECD 202 EEC C.2.	*Daphnia magna*/ Mobility (Test material 93.8 % Bifenthrin technical)	Static	48 h	0.056[1]	0.37[1]	-	1	Hooftman (2002)
OECD 202 EEC C.2.	*Cerodaphnia dubia*/ Mobility (Test material 93.8 % Bifenthrin technical)	Static	24 h	0.043[1]	0.31[1]	-	1	Hooftman (2002)
OECD 202 EEC C.2.	*Thamnocephales platyurus*/ Mobility (Test material 93.8 % Bifenthrin technical)	Static	24 h	0.032[1]	5.7[1]	-	1	Hooftman (2002)
OECD 202 EEC C.2.	*Hexagenia sp.*/ Mobility (Test material 93.8 % Bifenthrin technical)	Static	48 h	0.039[1]	0.39[1]	-	1	Hooftman (2002)
OECD 202 EEC C.2.	Caddisfly *sp.*/ Mobility (Test material 93.8 % Bifenthrin technical)	Static	48 h	0.031[1]	0.12[1]	-	1	Hooftman (2002)
OECD 202 EEC C.2.	***Gammarus pulex*/ Mobility** (Test material 93.8 % Bifenthrin technical)	**Static**	**48 h**	**0.032[1]**	**0.11[1]**	**-**	**1**	**Hooftman (2002)**

[1] Based on nominal concentrations (no measures of test concentrations were carried out)
[2] Based on mean-measured concentrations
[3] Based on the concentrations measured in the stock solutions (measured nominal concentrations)

Conclusion: Its proposed to retain as key study for the classification, the work of Surprenant (1985b) and Hooftman (2002): **EC_{50} = 0.11 µg.L^{-1}** (mean measured concentration), obtained with *Daphnia magna* and *Gammarus pulex*. Therefore, due to the EC_{50} value lower than 1 mg/l, it is consistent with classification as R50 or H400.

Long-term toxicity to aquatic invertebrates

Three studies are available for chronic toxicity to invertebrates. Two reproduction studies were performed under GLP following OECD 202[2], with *Daphnia magna* in a flow-through test design. The 21-d NOEC values are 0.0013 µg.L^{-1} (purity of test substance not specified) and 0.00095 µg.L^{-1} (both mean measured concentrations; test material: 10.36% ^{14}C-bifenthrin in hexane with radiopurity of 33.52 mCi/mM). In the second test, 58 offspring per parent animal survived, thus barely meeting guideline requirements (mean number of live offspring produced per parent animal surviving at test termination at least 60). However in the solvent control this validity criterion is fulfilled and overall the study can be considered as reliable. The third study was performed with *Mysidopsis bahia* in a flow through test design, following OECD 202[1] test guideline. The purity of

[2] Prior to publication of its revised version dated April 2004, OECD TG 202 covered both the 48h acute and the 21d reproduction test. The latter is also published as separate TG 211 since Sep 1998 (updated version Oct 2008).

the test material was not specified. The 21-d NOEC was 0.0012 $\mu g.L^{-1}$ (mean measured concentration).

Conclusion: The chronic toxicity of bifenthrin to invertebrates is very high.

7.1.1.3 Algae and aquatic plants

A study was performed on *Chlorella pyrenoidosa* and *Scenedesmus acutus*, following the OECD guideline 201 (purity not specified). This test was however invalidated. Indeed, the use of 0.1% acetone as solvent was deleterious to the growth rate of algae, and there was no effect of bifenthrin on growth rate above that observed in the solvent control.

Conclusion: No acceptable data are available.

7.1.1.4 Sediment organisms

A study under GLP revealed that bifenthrin has a high toxicity to *Chironomus riparius* larvae in a spiked water phase test (purity not specified). The 28-d LC_{50} was 3.96 $\mu g.L^{-1}$ and the 28-d NOEC was 0.32 $\mu g.L^{-1}$. In a spiked sediment test the 10-d EC_{50} was > 2500 $\mu g.kg^{-1}$, the EC_{50} for growth was 780 $\mu g.kg^{-1}$ sediment and the 10 day NOEC for growth was 83 $\mu g.kg^{-1}$ (Kelly, 2002 ; Putt 2005).

7.1.1.5 Other aquatic organisms

No data available

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

Not relevant for this type of dossier.

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro organisms

Two studies are available for soil macro organisms toxicity assessment. The first study (under GLP) has been performed on the acute toxicity of bifenthrin to the earthworm *Eisenia fetida*. Earthworms were exposed to contaminated soils with bifenthrin technical (purity: 88.35%) during a period of 14 days, following OECD 207 guideline. The results of these studies showed that bifenthrin has an acute 14-d LC_{50} higher than 18.9 $mg.kg^{-1}$ soil and the corrected 14-d LC_{50} in standard European soil is higher than 6.426 $mg.kg^{-1}$ (Roberts and Hakin, 1985).

The second study is a 56 days reproduction study, performed with *Eisenia fetida*, according to ISO 11268-2 guideline (purity of tested substance not specified). The 56-d NOEC for reproduction

equal to 2.13 $mg.kg^{-1}$ in test conditions and the corrected 56-d NOEC for reproduction in standard European soil equal to 0.7242 $mg.kg^{-1}$ (Stäbler, 2002).

7.2.1.2 Toxicity to terrestrial plants

Bifenthrin tested as formulated product (purity not specified) has no effect on the emergence of seedlings of 4 dicotyledons and 2 monocotyledons, at a soil addition rate of 0.08 mg/kg dry soil weight. Since only one dose was tested it is noted that no true DT_{50} or NOEC can be defined.

7.2.1.3 Toxicity to soil micro-organisms

No data available

7.2.1.4 Toxicity to other terrestrial organisms

Toxicity to birds

No data available

Toxicity to other above ground organisms

No data available

7.2.2 Calculation of Predicted No Effect Concentration (PNEC_soil)

Not relevant for this type of dossier.

7.3 Atmospheric compartment

No data available

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic micro-organisms

A study under GLP showed little to no effect of bifenthrin (purity: 97.8%) on the respiration of activated sludge micro-organisms. However concentrations applied were higher than the solubility limit (214, 619, 1929 $mg.L^{-1}$) and were not measured during the test. The NOEC determined was > 1929 $mg.L^{-1}$.

Conclusion: no effect was detected at the solubility limit.

7.4.2 PNEC for sewage treatment plant

Not relevant for this type of dossier.

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC_oral)

Not relevant for this type of dossier.

7.6 Conclusion on the environmental classification and labelling

Aquatic Acute 1 (H400: Very toxic to aquatic life) (CLP Regulation) and N; R50/53 (Directive 67/548/EEC)

Aquatic Chronic 1 (H410: Very toxic to aquatic life with long lasting effects) (CLP Regulation) and N; R50/53 (Directive 67/548/EEC)

The acute and the long-term classification categories are applied independently, according to CLP Regulation.

Scientific evidence

According to the studies presented, biodegradation of bifenthrin is expected to be limited in sediment, water and soil matrices. Bifenthrin is hydrolytically stable in water. There was no information or comment during public consultation opposing this conclusion. RAC confirms on this basis that bifenthrin is not rapidly degradable under CLP-criteria.

Bifenthrin meets the criterion for bioaccumulation potential according to the CLP Regulation (BCF in fish of ≥ 500 L/kg) and DSD (BCF in fish of ≥ 100 L/kg). With several reliable fish bioaccumulation studies available, demonstrating BCFs well above the classification criterion, RAC considers the potential of bifenthrin to bioaccumulate as decisive for environmental classification. There was no information or comment during public consultation opposing this conclusion.

Summary of relevant ecotoxicological endpoints for classification

Acute toxicity to fish	96h-LC_{50} = 0.1 µg/L
Acute toxicity to invertebrates	48h-EC_{50} = 0.11 µg/L
Chronic toxicity to fish	76d-NOEC = 0.012 µg/L
Chronic toxicity to invertebrate	21d-NOEC = 0.00095 µg/L

The LC_{50} and EC_{50} values for fish and invertebrates are four orders of magnitude lower than 1 mg/L, respectively.

Comparison of available aquatic toxicity information with the criteria for each hazard category (Annex I to the CLP Regulation including the modifications in the criteria according the 2nd ATP)

Acute aquatic hazard

For bifenthrin the lowest fish effects value is a 96h LC_{50} = 0.0001 mg/L (mean measured concentration) in rainbow trout *Oncorynchus mykiss*. Based on this low effect concentration RAC confirms the classification Category Acute 1 (H400) as adequate, and as 0.00001 mg/L < $E(L)C_{50}$ ≤ 0.0001 mg/L, a factor of M = 10 000.

- **Category Acute 1 (H400), M-factor (Acute) = 10 000**

Long-term aquatic hazard

For bifenthrin the lowest chronic aquatic effect value is a NOEC of 0.00095 µg/L (mean measured concentration) in a 21d reproduction test with the water flea *Daphnia magna*. This value is far below the set threshold (for non-rapidly degradable substance) of 0.1 mg/L.

Taking into account all the information on aquatic chronic toxicity and being not rapidly biodegradable, bifenthrin belongs to Category Chronic 1. The lowest chronic toxicity value (NOEC) ranging 0.0000001 < 0.00000095 ≤ 0.000001 mg/L, results for non-rapidly degradable substance in an M-factor (Chronic) = 100 000.

This suggestion takes into account that although there is no valid chronic test available with algae or aquatic plants, the specific action of synthetic pyrethroids like bifenthrin justifies to rely on the available fish and invertebrate test data for this conclusion. Thus RAC proposes the following classification

Category Chronic 1 (H410), M-factor (Chronic) = 100 000

Classification under DSD-criteria

As proposed by the dossier submitter, RAC confirms a classification as **N; R50/53** adequate, as bifenthrin is not rapidly biodegradable, expected to be stable in water and has a potential for bioconcentration in aquatic organisms.

In addition, as the 96h-LC_{50} value of 0.1 µg/L for fish is 0.00001 mg/L < $E(L)C_{50}$ ≤ 0.0001 mg/L, SCL are proposed as follows:

Specific concentration limits:

C ≥ 0.0025 %	N; R50/53
0.00025 % ≤ C < 0.0025 %	N; R51/53
0.000025 % ≤ C < 0.00025 %	R52/53

Apart from several technical comments, the public consultation expressed unitary support for the proposed classification. RAC confirmed the underlying scientific justification.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

Bifenthrin was evaluated in the context of the Biocidal Product Directive (98/8/EC) and it is therefore a requirement to harmonise classification for all endpoints.

OTHER INFORMATION

The information available was submitted in the scope of the Biocidal Product Directive for inclusion of the active substance bifenthrin in annex I of directive 98/8/CE.

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