Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling at Community level of

bifenthrin

ECHA/RAC/DOC No CLH-O-0000001740-81-01/F

Adopted

24 May 2011

Annankatu 18 | P.O. Box 400 | 00121 Helsinki | Finland
www.echa.eu | Tel.: + 358 9 68.61.80

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

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

Substance Name: ***bifenthrin***

EC Number: ***not allocated***

CAS Number: ***82657-04-3***

The proposal was submitted by ***France***
and received by RAC on ***22 February 2010.***

Harmonised classification originally proposed by the dossier submitter:

	Regulation (EC) No 1272/2008	Directive 67/548/EEC
Current entry in Annex VI of CLP Regulation (EC) No 1272/2008	None	None
Proposal by dossier submitter for consideration by RAC	Carc.2 – H351 Acute Tox. 3 – H331 Acute Tox. 3 – H301 STOT RE 1 – H372 Skin Sens. 1 – H317 Aquatic. Acute 1– H400 Aquatic. Chronic 1 – H410	Carc. Cat 3; R40 T; R23/25 Xn; R48/22 R43 N; R50/53
Resulting harmonised classification (future entry in Annex VI of CLP Regulation) as proposed by dossier submitter	Carc.2 – H351 Acute Tox. 3 – H331 Acute Tox. 3 – H301 STOT RE 1 – H372 Skin Sens. 1 – H317 Aquatic. Acute 1– H400 Aquatic. Chronic 1 – H410	Carc. Cat 3; R40 T; R23/25 Xn; R48/22 R43 N; R50/53

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/consultations/harmonised_cl/harmon_cl_prev_cons_en.asp*** on ***22 February 2010***. Parties concerned and MSCAs were invited to submit comments and contributions by ***8 April 2010***.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: ***Norbert Rupprich***
Co-rapporteur, appointed by RAC: ***Hans-Christian Stolzenberg***

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

The RAC opinion on the proposed harmonised classification and labelling has been reached on ***24 May 2011,*** in accordance with Article 37 (4) of the CLP Regulation, giving parties concerned the opportunity to comment. Comments received are compiled in Annex 2.

The RAC Opinion was adopted by ***consensus.***

OPINION OF RAC

The RAC adopted the opinion that bifenthrin should be classified and labelled as follows:

Classification & Labelling in accordance with the 2nd ATP to the CLP Regulation:

Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
				Hazard Class and Category Code(s)	Hazard state-ment Code(s)	Pictogram, Signal Word Code(s)	Hazard state ment Code(s)	Suppl. Hazard statement Code(s)		
	bifenthrin	–	82657-04-3	**Carc. 2** **Acute Tox. 3** **Acute Tox. 2** **STOT RE 1** **Skin Sens. 1B** **Aquatic. Acute 1** **Aquatic Chronic 1**	**H351** **H331** **H300** **H372** (nervous system) **H317** **H400** **H410**	**GHS06** **GHS08** **GHS09** **Dgr**	**H351** **H331** **H300** **H317** **H372** **H410**		**Acute M= 10 000** **Chronic M= 100 000**	

Classification & labelling in accordance with Directive 67/548/EEC:

Index No	International Chemical Identification	EC No	CAS No	Classification	Labelling	Concentration Limits	Notes
	bifenthrin	–	**82657-04-3**	**Carc. Cat 3; R40** **T; R23/25** **Xn; R48/22** **R43** **N; R50/53**	**T, N** **R: 23/25-40-43-48/22-50/53** **S: 23-24-36/37-38-45-60-61**	**N; R50/53: C ≥ 0.0025%** **N; R51/53: 0.00025% ≤ C< 0.0025%** **R52/53: 0.000025% ≤ C < 0.00025%**	

SCIENTIFIC GROUNDS FOR THE OPINION

The following part of the Opinion Document essentially is a targeted summary of the corresponding Background Document. This summary mainly corresponds to the endpoint-related "summary and discussion" chapters of the Background Document. Thus this summary concentrates on the most important experimental results, the history of decision finding and the final RAC proposal. Compared to the Background Document, this Opinion Document does not contain any additional information.

Substance identity

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)

General aspects

The substance is not currently classified in Annex VI to the CLP Regulation.

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.

Hazard classes and categories

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)

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.

<u>Irritation</u>

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.

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.

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.

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 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.

RAC considers that bifenthrin should be allocated to subcategory 1B (>= 30% responding animals at > 1% intradermal induction dose).

Repeated dose toxicity

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) NOAEL not 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.

<u>Mutagenicity</u>

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). Bifenthrine 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.

Carcinogenicity

The dossier submitter proposed to classify bifenthrin for carcinogenicity (CLP Carc. Cat 2 – H351, Carc. Cat. 3, R40 according to the Directive 67/548/EEC criteria). 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 in %	Control	50 ppm	200 ppm	500 ppm	600 ppm
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.

Carcinogenicity. Statistical decision criteria

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 have 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 opinion.

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

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

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 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 concordances 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.

RAC opinion on carcinogenicity of bifenthrin

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 classification 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 susceptibility 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. A classification as 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.

Toxicity for reproduction

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.

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.

Hazardous to the aquatic environment

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 $\geq$ 500 L/kg) and DSD (BCF in fish of $\geq$ 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.

Additional information

The Background Document, attached as Annex 1, gives the detailed scientific grounds for the Opinion.

ANNEXES:

Annex 1 Background Document (BD)[2]

Annex 2 Comments received on the CLH report, response to comments provided by the dossier submitter and rapporteurs' comments (excl. confidential information)

[2] The Background Document (BD) supporting the opinion contains scientific justifications for the CLH proposal. The BD is based on the CLH report prepared by a dossier submitter. The original CLH report may need to be changed as a result of the comments and contributions received during the public consultation(s) and the comments by and discussions in the Committees.