Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Bupirimate

EC number: 255-391-2
CAS number: 41483-43-6

CLH-O-0000001412-86-17/F

Adopted

06 June 2014

06 June 2014

CLH-O-0000001412-86-17/F

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

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

Chemicals name: **Bupirimate**

EC number: **255-391-2**

CAS number: **41483-43-6**

The proposal was submitted by **The Netherlands** and received by the RAC on **26 June 2013.** All classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS); the notation of 67/548/EEC, the Dangerous Substances Directive (DSD) is no longer given.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **02 July 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **16 August 2013**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by the RAC: **Norbert Rupprich**

Co-Rapporteur, appointed by the RAC: **Katalin Gruiz**

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

The RAC opinion on the proposed harmonised classification and labelling was reached on **06 June 2014** and the comments received are compiled in Annex 2.

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

OPINION OF THE RAC

The RAC adopted the opinion on **Bupirimate** that should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)	
Current Annex VI entry	No current Annex VI entry									
Dossier submitters proposal	612-288-00-0	bupirimate ISO); 5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate	255-391-2	41483-43-6	Carc. 2 Skin Sens. 1B Aquatic Chronic 1	H351 H317 H410	GHS07 GHS09 Wng	H351 H317 H410		M (chronic) = 1
RAC opinion	612-288-00-0	bupirimate ISO); 5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate	255-391-2	41483-43-6	Carc. 2 Skin Sens. 1B Aquatic Chronic 1	H351 H317 H410	GHS08 GHS07 GHS09 Wng	H351 H317 H410		M = 1
Resulting Annex VI entry if agreed by COM	612-288-00-0	bupirimate ISO); 5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate	255-391-2	41483-43-6	Carc. 2 Skin Sens. 1B Aquatic Chronic 1	H351 H317 H410	GHS08 GHS07 GHS09 Wng	H351 H317 H410		M = 1

SCIENTIFIC GROUNDS FOR THE OPINION

HUMAN HEALTH HAZARD ASSESSMENT

RAC general comment

RAC has not assessed respiratory sensitisation since no data were provided by the Dossier Submitter or during the Public Consultation.

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Bupirimate has no explosive properties and is not flammable under test conditions (respectively EEC A14 and A10 tests). The molecular structure, mass and composition of bupirimate do not indicate oxidizing properties. No relative self-ignition temperature could be determined. Bupirimate is not auto-flammable.

Therefore, the dossier submitter (DS) concluded that bupirimate does not warrant classification for physico-chemical properties.

Comments received during public consultation

Physical hazards were not specifically commented on.

Assessment and comparison with the classification criteria

RAC concluded that bupirimate does not fulfil the criteria for classification as explosive, flammable solid, self-reactive or pyrophoric substance, self-heating substance or oxidising solid and therefore classification for physical hazards is not warranted.

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

Based on the available acute toxicity studies, the DS did not propose to classify bupirimate for acute oral, dermal and inhalation toxicity.

Comments received during public consultation

These hazard classes were not specifically commented on.

Assessment and comparison with the classification criteria

Bupirimate was tested for acute oral, dermal and inhalation toxicity. For the evaluation of acute toxicity the dose levels resulting in lethality are relevant.

Oral
Bupirimate was tested for acute oral toxicity in the rat, mouse, guinea pig and rabbit. The highest dose level tested in all these species was 4000 mg/kg bw. The highest dose level at which lethality did not occur was 2000 mg/kg in the guinea pig and rabbit, 3200 mg/kg in the rat and 4000 mg/kg in the mouse. Only LD_{50} values below 2000 mg/kg result in classification for acute oral toxicity.

Dermal
Bupirimate was tested for acute dermal toxicity in the rabbit at a single dose level of 2000 mg/kg. No mortalities were observed. Only LD_{50} values below 2000 mg/kg result in classification for acute dermal toxicity.

Inhalation
In the only reliable inhalation study available (rat) the highest attainable concentration was 1340 mg/m³ (1.34 mg/L). This air-borne concentration did not result in lethality. Substances should not be classified if the inhalation LC_{50} is beyond 5000 mg/m³ (5.0 mg/L, dusts and mists).

Therefore, RAC supported the proposal of the DS not to classify bupirimate for acute oral, dermal or inhalation toxicity.

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

Summary of the Dossier submitter's proposal

Based on the assessment of the non-lethal adverse effects caused by bupirimate in the acute toxicity studies, the DS did not propose a classification for specific target organ toxicity (single exposure).

Comments received during public consultation

This hazard class was not specifically commented on.

Assessment and comparison with the classification criteria

Both in the acute dermal rabbit study (highest dose level of 2000 mg/kg) and in the acute rat inhalation study (highest air-borne concentration of 1340 mg/m³) no adverse effects were reported.

For the acute oral toxicity tests, dose-response data showed transient effects at relatively high oral dosages, but they did not indicate specific target organ toxicity after single exposure. Thus, the criteria for category 1 or 2 of STOT SE are not considered to be fulfilled. Furthermore, oral toxicity testing did not result in narcotic effects, thus the criteria for STOT SE, category 3 are also not met. In the acute dermal and inhalation toxicity studies no adverse effects were reported.

RAC therefore supported the conclusion of the DS for non-classification for specific target organ toxicity – single exposure.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

Based on the negative result of a standard rabbit skin irritation study the DS did not propose to classify bupirimate for skin corrosion/irritation.

Comments received during public consultation

This hazard class was not specifically commented on.

Assessment and comparison with the classification criteria

A single dose of 500 mg of bupirimate was dermally applied to 3 male rabbits for 4h. None of the rabbits showed any signs of test substance related lesions at any of the evaluation times (DAR, 2009). RAC therefore supported the conclusion of the DS for non-classification of bupirimate for skin irritation.

RAC evaluation of eye corrosion/irritation

Summary of the Dossier submitter's proposal

Based on two eye irritation studies in rabbits the DS proposed not to classify bupirimate for eye irritation. In the first study (Henderson, 1981, reliable with restrictions) very slight eye irritation

was reported; in the second study (Leuschner, 2001, reliable without restrictions) the substance was considered non-irritating.

Comments received during public consultation

One Member State competent authority (MSCA) supported the non-classification of bupirimate for eye irritation.

Assessment and comparison with the classification criteria

In the first eye irritation study in rabbits (Henderson, 1981) treatment caused moderate pain followed by slight redness of the conjunctivae and slight to mild chemosis with some discharge. All of the test eyes appeared normal after three days. No scores were reported (both in the CLH report and the DAR). Bupirimate was considered very slightly irritating for the eyes.

In the second eye irritation study in rabbits (Leuschner, 2001) the application of bupirimate did not cause any changes to the eyes of rabbits. Cornea opacity, iritis and redness and chemosis of the conjunctivae scored 0 at all time points (DAR, 2009).

RAC therefore supported the conclusion of the DS that bupirimate should not be classified for eye irritation.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

Based on weight of evidence which took into account a negative local lymph node assay (LLNA) and a positive Guinea Pig Maximisation Test (GPMT), along with one case of human sensitisation, the DS proposed to classify bupirimate for skin sensitisation.

The DS judged the GPMT as positive because the challenge concentration of 75% w/w as the highest non-irritating concentration, was considered adequate. In addition, the resulting sensitisation rates were higher than the GPMT-specific cut-off level for classification.

Comments received during public consultation

During public consultation two MSCAs supported the classification proposal. Industry questioned whether the result of the GPMT should be considered positive. Industry argued that the 75% challenge concentration in the GPMT could not be considered a highest non-irritating concentration and that the decline of response (24 days vs. 48 days post-challenge) in treated animals could be also due to a primary skin irritation.

Assessment and comparison with the classification criteria

For bupirimate there were two key skin sensitisation studies available: a GPMT (similar to OECD 406) and a LLNA test (OECD 429).

GPMT
In the GPMT, two challenge concentrations (30% and 75%) were tested. The intradermal induction concentration was 10%, the topical induction concentration was 75%. The test Guideline requires that the experimental animals are challenged with the highest non-irritating concentration. The study results indicated that at least the 75% challenge concentration resulted in slight primary skin irritation in controls.

Because of these skin reactions in the control group the overall grading of skin reactions (both in the control and test group) becomes important. Grading of skin reactions in the GPMT are shown in the table below (based on DAR):

Group	Conc. (w/w)	24 h after end of challenge				48 h after end of challenge			
		1	2	3	Total	1	2	3	Total
Control (10)	30%	1 10%	-	-	1 10%	-	-	-	- 0%

Group	Conc. (w/w)	24 h after end of challenge				48 h after end of challenge			
		1	2	3	Total	1	2	3	Total
Test (20)	30%	8 40%	1 5%	-	9 45%	3 15%	1 5%	-	4 20%
Control (10)	75%	2 20%	-	-	2 20%	-	-	-	- 0%
Test (20)	75%	5 25%	7 35%	2 10%	14 70%	3 15%	5 25%	-	8 40%

Uncertainty as to the interpretation of challenge reactions can arise when skin reactions are also seen in control animals. Because these skin reactions in the control groups result from primary skin irritation, there is some doubt as to the nature of reactions in the test groups. Furthermore (with reference to textbooks on cutaneous toxicity) a rapid fading of a challenge reaction would also suggest irritation rather than sensitisation (as seen in this GPMT).

In this situation one approach for defining the percentage of test animals showing hypersensitivity might be to subtract the percentage of control animals with a defined grading of skin reactions from the percentage of test animals with the corresponding grading of erythema. If there were 10% grade 2 and 90% grade 1 reactions in the test group and 80% grade 1 reactions in the control, the sensitisation response could be calculated as 20% (10% + [90%-80%]). This approach of calculating the sensitisation response in the GPMT is shown in the table below:

Challenge (concentration of substance in vehicle in % w/w)	Corrected sensitisation rate [%] 24 h after challenge	Corrected sensitisation rate [%] 48 h after challenge
75%	50% (25% - 20% + 35% + 10%))	40% (15% + 25%)
30%	35% (40% - 10% + 5%)	20% (15% + 5%)

Due to the 20% irritation rate in the controls the challenge concentration of 75% can be questioned as a non-irritating concentration; it seems that the 30% challenge concentration is more adequate (although there was still a 10% incidence of slight primary irritation). Furthermore, there was a reversion of response both in the control and the treated animals from 24 h to 48 h following end of challenge. This reversion of response might be more characteristic of a primary irritation reaction compared to the time course of skin reactions based on sensitisation. Depending on the challenge concentration chosen and the time point of observation preferred, the sensitisation rate ranged from 50% (highest challenge concentration, observation after 24 h) to 20% (lowest challenge concentration, observation after 48 h). Accounting for the above-mentioned considerations the GPMT indicates a weakly positive sensitisation response at most.

LLNA

The skin sensitisation potential was also tested in the LLNA. In this study an EC3 value could not be calculated because none of the tested bupirimate concentrations induced a stimulation index above the threshold of 3 (see Table below). No signs of systemic toxicity were observed. All animals showed slight erythema (score 1) of the ear up to a concentration of 25%; at the high-concentration level of 50%, well defined erythema (score 2) was observed.

LLNA (OECD 429)	**Induction** [%]			**Observation**	
	topical 1 day 1	topical 2 day 2	topical 3 day 3	Stimulation Index (SI)	EC3

LLNA (OECD 429)	Induction [%]			Observation	
	topical 1 day 1	topical 2 day 2	topical 3 day 3	Stimulation Index (SI)	EC3
treatment group 1	10%	10%	10%	2,26	No EC 3 because of SI lower than 3
treatment group 2	25%	25%	25%	1,84	
treatment group 3	50%	50%	50%	1,52	

Thus RAC concluded that bupirimate does not show skin sensitising properties in the LLNA. Bupirimate has been manufactured and used in the UK for the last 25 years. During this period one case of skin sensitisation (confirmed by patch testing) was documented involving a formulation worker.

Classification of bupirimate has to be essentially based on the 2 (conflicting) results of the GPMT and LLNA. Both studies are regarded as acceptable studies. The reasons for the difference between the two studies are not known. The human evidence (one case documented) cannot sufficiently contribute to a conclusion on classification. According to the CLP Guidance, test results from the LLNA, GPMT and the Buehler assay can be used directly for classification. In addition, a substance may be classified as a skin sensitiser on the basis of a positive test result in one of the above described animal tests.

The 75% challenge concentration in the GPMT resulted in a corrected sensitisation rate of 50% and 40% at 24 h and 48 h, respectively. Both sensitisation rates exceed the cut-off level of 30%, thus the GPMT is considered positive. Although the 75% challenge concentration caused primary irritation in the controls, it needs to be emphasised that there were only grade 1 skin erythema in the controls whereas in the test groups erythemas reached grades 2 and 3. There was only a partial fading out of skin reactions from the first to the second time point of observation; without further data it should be assumed that at least the skin reactions observed at the second time point of observation are related to skin sensitisation. RAC acknowledged that the positive response in the GPMT is rather weak.

RAC concluded that the negative LLNA does not override the weakly positive GPMT. Based on the negative LLNA and the weakly positive GPMT, RAC threrefore supported the proposal to classify bupirimate for skin sensitisation (DSD and CLP).

According to the CLP Guidance, skin sensitisers shall be classified in the general category 1 where data are not sufficient for sub-categorisation. Based on the data available, RAC however considered the experimental evidence sufficient for placing the substance in a subcategory: In the GPMT an intradermal induction concentration of 10% resulted in a maximum sensitisation rate of 50%. Thus it may be assumed that an intradermal induction concentration of 1% will not result in a sensitisation rate of higher than 60% (the condition for subcategory 1A).

RAC considered bupirimate as being a moderate skin sensitiser of subcategory 1B.

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

Summary of the Dossier submitter's proposal

The CLH report contained a detailed description and assessment of the bupirimate data on repeated dose toxicity (RDT). Sufficiently reliable repeated dose toxicity studies after oral administration were available for rats and dogs. The DS compared the available RDT data with the DSD and CLP classification criteria. The DS concluded that the available oral toxicity studies do not show significant toxic effects at dose levels requiring classification as STOT RE.

Comments received during public consultation

3 MSCAs indicated general agreement with the classification proposal; however no specific comments were received on repeated dose toxicity.

Assessment and comparison with the classification criteria

In order to get a systematic overview on the bupirimate information critically relevant for STOT RE classification, study-specific cut-off levels, the dose range tested and the most relevant results are presented in the following tables for rats and dogs.

Rat
The effects observed after oral administration of bupirimate in rats do not require classification for STOT RE. The only effect observed below the cut-off level for STOT RE 2 is incontinence in rats (at 250 mg/kg/d in the 10-day oral rat study). Urinary incontinence was reported in the acute toxicity studies but not in the longer-term rat studies. In conclusion, the incontinence observed might be considered an acute effect rather than an effect triggered by repeated exposure.

Dog
The effect on the testis in the 90-day oral dog study is not considered a relevant adverse effect (most of the males were still juveniles at the end of the study).

In the 90-day oral dog study bupirimate administration resulted in thymus toxicity. At the dose level of 30 mg/kg/d (below the cut-off level for STOT RE 2 of 100 mg/kg/d) there was a statistically significant reduction of absolute (not relative) thymus weight only in females. Thymus weight reduction was more pronounced at 600 mg/kg/d. There was no reporting of specific histopathological changes in the thymus. In the 2-year dog study (highest dose of 200 mg/kg/d compared to the cut-off level of 12.5 mg/kg/d) there was neither a statistically significant reduction in thymus weight nor histopathological changes in the thymus. Without any indications for specific histopathological changes in the thymus in either of the dog studies, the significant thymus weight reduction in females at 30 mg/kg/d (in the 90-day study) does not warrant classification.

Overall, it can be concluded that in the available short- and longer term studies, no biologically relevant effects warranting classification under CLP have been observed. RAC therefore supported the proposal of the DS that bupirimate should not be classified for specific target organ toxicity upon repeated exposure.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Based on the results of *in vitro* and *in vivo* mutagenicity studies, the DS did not consider bupirimate a genotoxic substance. The DS proposed not to classify bupirimate for germ cell mutagenicity.

Comments received during public consultation

3 MS indicated a general agreement with the classification proposal; however no specific comments were received on germ cell mutagenicity.

Assessment and comparison with the classification criteria

The following table contains a summary of the bupirimate mutagenicity data. The table contains those mutagenicity studies which are considered sufficiently reliable. *In vitro* testing for DNA damage and gene mutations was negative. Positive results were obtained in the *in vitro* chromosome assay on human lymphocytes. However, clastogenicity was not seen in the *in vivo* micronucleus test, which was considered to overrule the positive chromosome aberration test *in vitro*. From these results it is concluded that bupirimate is not to be considered genotoxic.

	DNA damage	Gene mutation	Chromosome aberration
In vitro	DNA repair assay on human embryonic fibroblast cells: negative	Ames test: negative Gene mutation in mouse lymphoma cells L5178Y(TK): negative	Chromosome aberration assay on human lymphocytes: positive
In vivo	-	-	*In vivo* micronucleus test: negative Dominant lethal mutation assay in mice: negative

Overall, RAC supported the conclusion of the dossier submitter that bupirimate should not be classified for mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

Based on the results of a 24-month oral carcinogenicity study in rats, the DS proposed to classify bupirimate for carcinogenicity (Carc. Cat. 2, H351). The key evidence for the DS's classification proposal is a slight increase of subcutaneous fibromas in female rats at the highest dose level tested.

Comments received during public consultation

Relevant findings in the bupirimate rat carcinogenicity study were related to increased incidences of mammary gland adenocarcinomas, thyroid follicular adenomas and skin fibromas. There were no comments received during public consultation disagreeing with the DS's assessment that the increased incidences of mammary gland adenomacarcinomas and thyroid follicular adenomas do not warrant classification. In contrast, there were differing opinions as to the assessment of the skin fibromas in female rats. 4 MSCAs seemed to agree with the proposal to classify bupirimate for carcinogenicity. 1 MS and Industry disagreed with the DS's proposal. The following table contains a short overview on the pros and cons exchanged during public consultation (with specific reference to skin fibromas in female rats as a possible trigger for classification). Additional elements can be found in Annex 2.

Table: Comments received during public consultation related to subcutaneous fibroma data in the female rat

Topic	Comments proposing non-classification	Comments (mainly by the DS) in favour of classification
Statistically significant increase in skin fibromas up to 12.5 % and comparison with historical control data.	Low increase. No clear dose-response. Slightly above contemporary historical controls (beyond 9%). Within historical controls compiled in open literature (range up to 15%). Combined incidences in males and females within combined historical control incidences.	Incidences which are statistically significant and outside the upper range of relevant controls cannot be considered a coincidence. Absence of a very clear dose-response might be due to a broad dose-spacing and a high threshold. Historical control data from other laboratories less relevant. Combination of sex-specific

Topic	Comments proposing non-classification	Comments (mainly by the DS) in favour of classification
	Skin fibromas are considered as common (benign) lesions in aging rats.	incidences does not allow for the assessment of tumours induced through a sex-specific mechanism. It is acknowledged that skin fibromas are considered as common lesions in aging rats, however, this information is incorporated in the data from historical controls.
Long-term mouse and dog study	No corresponding neoplastic lesions in the mouse and dog study.	Methodological deficiencies of the mouse and dog studies impair their reliability.
Negative ethirimol (a metabolite) carcinogenicity study in rats	The missing evidence for carcinogenicity of a relevant metabolite of bupirimate supports non-classification	Ethirimol dose levels tested (up to 500 ppm) was much lower than dose levels of bupirimate with increased incidence of skin fibroma (5000 ppm). Another main metabolite (ethyl-guanidine) which is not formed from ethirimol has not been tested for carcinogenicity.
Mechanism of skin fibroma development	Occurrence of skin fibroma is not biologically plausible.	MOA for skin fibromas indeed not known, but a missing MOA is not a reason not to classify. Lack of dermal pre-neoplastic lesions in the 90-day studies as one of the reasons for Cat. 2 instead of Cat. 1B.
Bupirimate is not genotoxic	Non-genotoxicity is considered to be a trigger for non-classification	Various non-genotoxic substances are classified as carcinogens, thus missing mutagenicity is no trigger for non-classification.

Assessment and comparison with the classification criteria

The results of the 2-year oral studies in mice and Beagle dogs did not show any evidence for carcinogenicity of bupirimate. The mouse study however was considered not acceptable (due to severe limitations in study design). The dog study cannot be considered a carcinogenicity study. The carcinogenicity assessment of bupirimate must therefore be based on the results of the rat carcinogenicity study.

The following table presents selected rat carcinogenicity data. Reporting of data is limited to mortality, body weight development and relevant neoplastic alterations (mammary tissue, skin and thyroid gland). There are no other treatment-related neoplastic lesions (CLH report or DAR).

Table: Selected rat carcinogenicity data

Dose	Control	100 ppm	1000 ppm	5000 ppm
Sex	**Females**			
Food consumption 0-78 wks in % of control		-	-	+6%

Dose	Control	100 ppm	1000 ppm	5000 ppm
Sex	**Females**			
Clinical signs	no	no	no	no
BW* at 78 wk in % of control	-	-8%	-6%	-34%
BWG* 0-78 wks in % of control	-	-9%	-8%	-40%
Cumulative mortality at week 78	11/40	8/40	8/40	13/40
Cumulative mortality at week 104	26/40	24/40	25/40	31/40
Mammary tissue: adenocarcinoma	1/40 2.5%	2/40 2,5%	2/40 5%	5/40 12.5%
Thyroid: Follicular adenoma	0/40	1/40	0/40	2/40
Skin: Subcutaneous fibroma	1/40 2.5%	1/40 2.5%	1/40 2.5%	5/40* 12.5%

* p<0.05 : Fisher's exact probability test

Dose	Control	100 ppm	1000 ppm	5000 ppm
Sex	**Males**			
Food consumption 0-78 wks in % of control		+5%	-4%	-9%
Clinical signs	no	no	no	no
BW at 78 wk in % of control	-	-	-6%	-16%
BWG 0-78 wks in % of control	-	-	-7%	-18%
Cumulative mortality at week 78	16/40	24/40	15/40	10/40
Cumulative mortality at week 104	37/40	40/40	30/40	29/40
Mammary tissue: adenocarcinoma	0/40	1/40	0/40	0/40
Thyroid: Follicular adenoma	1/40	2/40	5/40 (DAR)	11/40* (27.5%)
Skin: Subcutaneous fibroma	0/40	0/40	3/40 7.5%	5/40 12.5%

* p<0.05 : Fisher's exact probability test

Mortality and body weight gain
Even at the top dose levels no clinical signs were observed throughout the study (males, females). Survival was considerably below the required 50% in each group at the end of the study. There was no indication of a treatment-related effect on mortality. While body weight gain (78 weeks, medium dose level) was about minus 10%, at the top dose there was a minus 18% body weight gain in males, and an extremely high reduction of body weight gain (minus 40%) in the females.

According to the OECD guidance document on the conduct and design of chronic toxicity and carcinogenicity studies (No. 116, April 2012) the top dose level in a carcinogenicity study should provide a slight depression of body weight gain of not more than 10% without substantially altering normal life span due to effects other than tumours. Normal life span of animals was reduced by a high clearly non-treatment related mortality in all dose groups; a relationship between the high reduction in body weight gain at the high dose level and mortality cannot be established.

Mammary tissue
In females the incidence of adenocarcinomas was slightly elevated in the high dose group; however this small increase was not statistically significant. The high-dose incidence of 12.5% is

similar to the upper range of relevant historical control incidences but well beyond the corresponding median control incidences of 4.5 and 5.6% (see both Tables below). This small increase in mammary adenocarcinoma cannot be completely dismissed.

Dose	Control	100 ppm	1000 ppm	5000 ppm
Sex	**Females**			
Mammary tissue: adenocarcinoma	1/40 2.5%	2/40 5%	2/40 5%	5/40 12.5%

Historical control data for mammary adenocarcinoma in the SD rat:

Period and Number of studies	Laboratory	Male Incidences Ranges and average	Female Incidences Ranges and average	Reference
1975-1977 1977-1979 9-12 studies	Huntingdon		0 - 13.3% (4.5%) 3 - 13.3% (5.6%)	Supplementary information

Thyroid gland
A statistically significant increase of incidence of thyroid follicular adenoma was found in males only at the highest dose level of 5000 ppm in food (tumour incidences: 1/40, 2/40, 5/40, 11/40*). No follicular adenocarcinomas were detected.

The concern of rat thyroid tumours for humans depends on the specific mechanism of induction. The Guidance on the Application of the CLP criteria specifically considers rat thyroid tumours of insufficient concern for humans if they are mediated by liver UDP glucuronyltransferase induction. In the corresponding Specialised Expert's opinion (ECBI/49/99-Add.1 Rev.2) a more generalised recommendation was given: *If the disturbance of the thyroid-pituitary axis can be shown (based on different specific mechanisms) and if it is a low or medium potency substance, then no classification is warranted. Based on the T25 concept bupirimate should be considered a low potency substance; the T25 (incidence of 27.5% at the high dose level of 5000 ppm) for thyroid adenomas is higher than the cut-off level of 100 mg/kg/d*. Thus, according to the "Specialised Experts" there should be no classification for bupirimate if there is sufficient evidence for a thyroid hormone imbalance.

In a 10-day oral toxicity study in rats relative liver weight in males was increased at the dose levels of 250 mg/kg/d (+21%) and 1000 mg/kg/d (+52%) (table 15 of CLH report). In the 90-day oral toxicity study in rats there was no influence on liver weight while there was an increase of the absolute thyroid weight of up to 20% (for details see table 17 of CLH report).

In addition there was a thyroid function mechanistic study (male rats, oral, daily for 28 days) including the relevant dose level of 5000 ppm and a high dose level of 20 000 ppm. T4 reduction was dose-related (-20% at 5000 ppm and –35% at 20 000 ppm). The TSH level was increased at 5000 ppm, but not at 20 000 ppm. I^{125} uptake was higher in the test groups (nearly 2-fold). However, the thyroid weight data presented in the table of the CLH report appear different compared to the original table in the DAR where there seems to be a dose-related increase of thyroid weight of up to 43%. Histopathology data in the test groups indicate follicles which seem to be more active (less colloid, hypertrophy of follicle cells, increased rate of mitosis). Thus, overall, this thyroid function study gives some evidence that bupirimate affects the thyroid hormone axis. However, there is no convincing evidence for a specific mechanism resulting in this hormonal perturbation. Specific thyroid toxicity via liver enzyme induction has not been completely verified.

Overall, RAC concluded that the increased incidence of thyroid gland adenomas in male rats is not sufficient for classification, mainly because there were only benign tumours, the corresponding potency was low and there was some evidence of perturbation of the pituitary-thyroid gland axis after administration of bupirimate.

Skin
In female rats the increase in the incidence of subcutaneous fibroma was statistically significant (tumour incidences: 2.5, 2.5, 2.5, 12.5%).

In males there was evidence of a dose-related trend in the incidences of skin fibroma (tumour incidences: 0-0-7.5-12.5%). However, no statistical significance was indicated (although the difference in incidence between the control and top dose level was higher than in the females) (see the Table below).

Table: Subcutaneous fibromas in the SD rat bupirimate carcinogenicity study

Dose	Control	100 ppm	1000 ppm	5000 ppm
	Females			
Skin: Subcutaneous fibroma	1/40 2.5%	1/40 2.5%	1/40 2.5%	5/40* 12.5%
	Males			
Skin: Subcutaneous fibroma	0/40	0/40	3/40 7.5%	5/40 12.5%

Overall, historical control incidences for females were lower than for males. The use of historical control data from other laboratories (Charles River Laboratories, 2004 and Baldrick 2005) in particular for studies conducted at different time periods, severely limit their value for comparison with the bupirimate data.

For bupirimate, contemporary historical control data from the same laboratory (Huntingdon Life Sciences) are available. The Bupirimate study was reported in 1976; the annual details for the historical control data represent the start of the studies. The best temporal match is with the oldest Huntingdon data (studies started during the years 1975-1977).

The 12.5% incidence of skin fibroma in females is outside the highest upper range of the three contemporary historical control data sets in the same laboratory. Moreover, the average historical control incidences are only around 3%. Thus the tumour incidence in the concurrent female controls is consistent with the historical control data. Based on these experimental and historical data, the small increase of skin fibromas in female rats should be considered treatment-related.

Historical control incidences for male rats are higher than for female rats, with average values of about 10% (for the years 1975 to 1979) but markedly less in the period from 1973 to 1974 (average value not reported). Overall it is recommended to put some more weight on the assessment of the female rat data, without however totally disregarding the male data.

Table: Historical control data for skin fibromas in the SD rat

Period and Number of studies	Laboratory	Male Incidences Ranges and average	Female Incidences Ranges and average	Reference
1973-1974 1975-1977 1977-1979	Huntingdon	0 - 5.7% (?%) 0 - 24.3% (8.7%) 0 - 20.3% (10.3%)	0 - 4.3% (?%) 0 - 6.8% (3.1%) 0 - 9.0% (2.8%)	CLH report and supplementary information

Period and Number of studies	Laboratory	Male Incidences Ranges and average	Female Incidences Ranges and average	Reference
9-12 studies				
1989-2002 31 studies	Various laboratories	0 – 11% (4%)	0 – 4% (0.6%)	Charles River Laboratories (2004)
1991-2002 13 studies with dual controls	Covance Lab	6.2 – 41.7% (25.1%) 3.1 – 41.7% (20.2%)	0 – 15.0% (5.6%) 0 – 8.3% (2.4%)	Baldrick (2005)

Overall conclusion for carcinogenicity
The benign tumours in the thyroid gland (males) are not considered sufficient evidence for classification.

Although the increased incidence of carcinomas in the mammary gland (females) is not statistically significant, the corresponding high-dose incidence and the comparison to relevant historical controls indicate that relevance of these mammary adenocarcinomas cannot be completely dismissed.

The increased incidence of subcutaneous fibromas in females (with some supportive evidence in males) indicates sufficient concern for classification:

- With reference to the historical control data available the increased incidence of skin fibromas in female rats at the high dose level is considered treatment-related.
- It should be noted that this high dose level is compromised by a rather high reduction of body weight gain (minus 40%). This raises the question whether the maximum tolerated dose is exceeded. However, this reduced body weigth gain is not accompanied by any clinical effects, or excessive toxicity to the skin.
- There is a corresponding increase in subcutaneous fibroma in male rats as well, which might be considered at least supportive evidence (the historical control incidences in male rats are higher than in female rats)
- Because of severe limitations in study design, the negative results in the long-term mouse and dog studies should be given less weight than the female rat carcinogenicity data.
- The main metabolite ethirimol was found to be negative for carcinogenicity; however the dose level tested (500 ppm) was one order of magnitude below the active bupirimate dose level (5000 ppm). Thus the negative findings for ethirimol cannot sufficiently counter the slightly positive bupirimate study.
- Bupirimate is not considered to be genotoxic; there is no information on a possible mode of action for the development of the subcutaneous fibromas in the high dose group of female rats.
- Bupirimate only induced benign subcutaneous tumours. There is no additional general information in the CLH report on the possible malignancy of subcutaneous fibromas in female rats.

According to CLP a substance should be classified in Category 1B if a causal relationship has been established between the agent and an increased incidence of malignant neoplasms or of a combination of benign and malignant neoplasms in at least two species or in two independent studies in one species. Substances may also be classified in Category 1B according to CLP if they produce an increased incidence of tumours in both sexes of a single species in a well-conducted study or if the substance leads to an unusual degree of malignant of neoplasms in one species and sex.

For bupirimate the carcinogenicity findings are not considered to fulfill these conditions; RAC is of the opinion that the bupirimate data do not allow for a classification in CLP Category 1B.

If there is limited evidence of carcinogenicity in animal studies, classification as Category 2 carcinogen or even no classification is possible.

- There is a small increase in mammary adenocarcinomas in females. Although this increase is not statistically significant, it cannot be completely dismissed.
- In the rat there is a spontaneous occurrence of skin fibromas. However, based on the dose-response data for bupirimate and the contemporary historical control incidences the increase in subcutaneous fibromas in female rats should be considered treatment-related. The effective dose level resulted in a marked reduction of body weight gain as well, but there was no parallel substance-related excessive toxicity and no indication of a specific effect on the skin which might have been the cause for the induction and development of the skin fibromas. The MOA is not known, thus irrelevance of the tumours for humans cannot be assumed.

Weighing the data available, RAC concluded that a carcinogenicity classification of bupirimate is more appropriate than no classification. Because there is only limited evidence for carcinogenicity, RAC supported the conclusion of the dossier submitter that bupirimate should be classified as a Category 2 carcinogen (Carc. 2, H351) under CLP.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

Based on the results of two developmental toxicity studies conducted in rats and rabbits, (oral gavage) and a two-generation reproduction study in rats (feeding study) the DS concluded that there was no evidence of reproductive toxicity of bupirimate (both for effects on fertility and developmental toxicity). The DS proposed not to classify bupirimate for reproductive toxicity.

Comments received during public consultation

3 MSCAs indicated general agreement with the classification proposal; however no specific comments were received on reproductive toxicity (development or fertility).

Assessment and comparison with the classification criteria

The reproductive toxicity assessment of bupirimate is based upon the results of 3 toxicity studies: 2 developmental toxicity studies (rats and rabbits) and 1 multigeneration rat study.

The results from the multigeneration study in rats are minimally reported (both in the CLH report and DAR). Based on the data available there is no convincing indication that bupirimate affected the 3 generations differently, so that it is considered justified to give an overview of parental toxicity, fertility impairment and pup toxicity that is valid for all 3 generations tested: parental toxicity is characterised by an about 10% reduction of body weight in males and females at the high dose level (200 mg/kg/d). There was no indication of fertility impairment (mating, fertility, gestation). Changes in pup parameters essentially occurred at the high dose level: there was a reduction in pup body weight (PND 25) up to about 20%, and a change of parameters (e.g. eye opening time) indicating delayed physical development in pups (no quantitative data). The conclusion in the CLH report is that there is no evidence that pups were more sensitive than adults.

Developmental toxicity was examined in Wistar rats given bupirimate (by gavage, from gestational days 7 to 16). The dose levels administered were 0, 50, 150 and 400 mg/kg/d. Maternal toxicity was observed in a few animals at the lowest dose, it became evident at the mid and high dose levels (salivation, urinary incontinence, decreased food consumption and decreased body weight gain). Litter responses (number, growth and survival of foetuses *in utero*) were within normal limits; the only exception was a change in the sex ratio at the high dose (43% males compared to about 55% in the other groups including the control). The DS did not consider this change of sex ratio to be treatment-related because of the absence of increases in embryonic deaths. Historical control data indicate that mild shifts in the male to female sex distribution ratio often occur, and little significance is placed on values that fall within normally expected ranges e.g. 44% to 56%. Examination of foetuses did not reveal external, visceral or skeletal malformations.

Minor skeletal defects (delayed ossification) were detected at the mid and high dose levels. Taken together, both maternal toxicity and minor skeletal effects (delayed ossification) were evident at the two highest dose levels. Based on these results the developmental toxicity study in rats does not show sufficient evidence in support of classification.

In the teratogenicity study with rabbits (New Zealand White) bupirimate was administered at daily oral dosages of 20, 80 and 320 mg/kg/d (by gavage, from gestational days 6 to 28). Adverse effects are limited to the high dose level. At this high dose level some degree of maternal toxicity (reduced food consumption and body weight gain) and a slight increase of abortions (0/22, 0/22, 1/22 and 2/22) was observed. No treatment-related effect occurred on the litter parameters. From examinations of the foetuses no treatment-related external or visceral malformations were reported. There was however an increase in skeletal malformations (2/166, 4/188, 4/177 and 11/174). This increase was mainly attributed to one litter with 5 foetuses presenting eight lumbar vertebrae. It was reported that this skeletal malformation is known to occur spontaneously (no further data provided). No other malformations (type of malformations not reported) showed a dose-response relationship, were considered to be spontaneous or were within the range of historical control data. At the high dose there was an increased incidence of slightly impaired ossification in some bones and of 13 ribs (full supernumerary ribs). Based on these results (skeletal deviations at the highest dose level with maternal toxicity) the teratogenicity study in rabbits does not indicate sufficient evidence for classification.

Fertility impairment
Based on no effects on the fertility parameters in the oral rat multigeneration study, RAC supported the conclusion of the DS that bupirimate should not be classified for effects on fertility.

Developmental toxicity
The assessment of the developmental toxicity potential of bupirimate is based on the results of the rat multigeneration study and the developmental toxicity studies in rats and rabbits. In the multigeneration study there was some pup toxicity, but the pups were not considered more sensitive than parental animals. In the developmental toxicity studies with rats and rabbits no treatment-related malformations were reported; delayed ossification occurred at maternally toxic dose levels both in rats and rabbits. Based on these data, RAC supported the conclusion of the DS that bupirimate should not be classified for developmental toxicity.

In conclusion, RAC agreed with the DS that classification of bupirimate for reproductive toxicity is not warranted.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

The DS proposed to classify bupirimate as Aquatic Chronic 1 (H410) with a chronic M-factor of 1.

The lowest L(E)C_{50} value for bupirimate was between 1.0 and 1.5 mg/L and this was measured in a fish study. No mortality was observed at 1.0 mg/L, whereas all the fish died at 1.5 mg/L, suggesting a steep dose-response curve. In a second fish study an LC_{50} value between 1.25 and 2.5 mg/L was obtained. Based on the lowest LC_{50} value between 1.0 and 1.5 mg/L, bupirimate does not fulfil the criteria for classification as acutely toxic to the aquatic environment.

The DS considered bupirimate as not readily biodegradable since 25.5% degradation was achieved after 28 days in a ready biodegradability test conducted according to the OECD301 B guideline. In a water/sediment degradation simulation test, the average DT_{50} of the whole system was 42.3 days. Mineralisation was less than 3% after 120 days. Bupirimate was hydrolytically stable with a DT_{50} value of >30 days. Bupirimate was photolysed rapidly in an aqueous solution with a DT_{50} value of 0.02 days. Based on these findings the DS considered bupirimate as being not rapidly degradable in the aquatic environment.

Bupirimate does not fulfil the criterion for bioaccumulation (BCF > 500), as the highest whole fish BCF derived for bupirimate was 185 L/kg (not lipid normalized).

The lowest NOEC, measured in a fish test, was 0.10 mg/L. The NOEC value of 0.10 mg/L falls within the range 0.01 < NOEC ≤ 0.1 mg/L. Being not rapidly degradable, the DS concluded that bupirimate therefore fulfils the criteria for classification as Aquatic Chronic 1 with a chronic M-factor of 1.

Comments received during public consultation

Four MSCAs expressed their agreement with the proposed environmental classification.

One MSCA brought up the problem of low pKa (acid dissociation constant) value of bupirimate (4.4) and its consequent dissociation in water. The DS clarified that ionic dissociation occurs mainly at a pH lower than 2.4, and that dissociation is not significant at a pH higher than 6.4. Therefore, in the normal pH range of aquatic habitats (between 6.0 and 9.0), bupirimate is found mainly in the undissociated form.

Assessment and comparison with the classification criteria

Acute aquatic hazards

Based on the results of the aquatic toxicity studies detailed in the DS section, RAC supported the conclusion of the DS that bupirimate does not fulfil the criteria for classification as acutely toxic to the aquatic environment.

Aquatic chronic hazards

Based on the information provided by the DS on ready biodegradability, simulation studies, hydrolysis and photolysis, RAC supported the conclusion of the DS that bupirimate is not rapidly degradable.

Moreover, bupirimate does not fulfil the bioaccumulation criterion of BCF > 500, as the lipid normalized BCF value was 80–128 L/kg. This value might be a conservative estimate, since the fish BCF test used a radiolabelled substance and, in absence of parent substance analysis, the measured BCF might include the metabolities.

RAC agreed with the DS conclusion regarding long term toxicity and concluded that, since the lowest NOEC value (0.10 mg/L) falls within the range 0.01< NOEC ≤0.1 mg/L and the substance is not rapidly degradable, it fulfils the criteria for classification as Aquatic Chronic 1 with a chronic M-factor of 1.

RAC noted that the NOEC value obtained from the static algae growth test (0.32 mg/L) is not reliable, since it is based on nominal concentrations and the substance shows a very rapid photolysis. This would suggest that a more realistic NOEC may have been lower. In addition, the growth rate of the control was not in the exponential growth phase for the whole testing period.

ANNEXES:

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

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