Committee for Risk Assessment

RAC

Annex 1

Background document

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

Bupirimate

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

CLH-O-0000001412-86-17/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

06 June 2014

CLH report

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name: Bupirimate

EC Number: 255-391-2

CAS Number: 41483-43-6

Index Number:

Contact details for dossier submitter:

RIVM/SEC, Bureau REACH

PO Box 1, 3720 BA Bilthoven. The Netherlands

bureau-reach@rivm.nl

Version number: 2 **Date:** May 2013

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	bupirimate
EC number:	255-391-2
CAS number:	41483-43-6
Annex VI Index number:	
Degree of purity:	≥ 94.5%
Impurities:	ethirimol ≤0.2% toluene ≤0.3% The impurities present do not affect the classification and labelling of bupirimate

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation		
Current proposal for consideration by RAC	Carc. 2 (H351) Skin sens Cat. 1B (H317) Aquatic Chronic 1 (H410) Chronic M-factor of 1	Carc Cat 3; R40 Xi; R43 N, R51/R53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 2 (H351) Skin Sens. 1B (H317) Aquatic Chronic 1 (H410) Chronic M-factor of 1	Carc Cat 3; R40 Xi, R43 N, R51/R53

1.3 Proposed harmonised classification and labelling based on CLP Regulation and/or DSD criteria

Bupirimate is a plant protection product which has been considered for inclusion in Annex I of Directive 91/414/EC. The classification has not been assessed before by RAC or TC-C&L. Therefore, RAC is requested to review all hazard classes including those hazard classes for which no classification is proposed (CLP article 36.2).

A review of the available toxicity data for bupirimate has revealed that classification for human health and environmental hazards in Annex VI of Regulation EC no.1272/2008 is relevant.

The 2nd ATP has implemented the 3rd revised edition of GHS in which classification and assignment of M-factors can also be based on chronic aquatic toxicity. Based on the criteria of the 2nd ATP, a classification and an M-factor based on the chronic aquatic toxicity is proposed.

According to Directive 67/548/EEC and Directive 1999/45/EC as amended by Directive 2006/8/EC, no distinction between acute and chronic SCLs can be made since only acute aquatic toxicity data are allowed for deriving classifications and SCLs. Therefore, only one set of SCL are proposed for classification of bupirimate according to DSD criteria.

Buprimate induced a small but statistically significant increase in skin fibroma in female rats that was outside the range of the historical controls. In males there was a comparable increase though not statistically significant and within the historical controls. There is no information on the mechanism. Therefore it has to be assumed that this type of tumour is relevant to humans. Classification as Carc. 2 (H351) and Carc Cat 3; R40 is proposed because the carcinogenic effect was only observed in one species and bupirimate is not genotoxic.

In accordance with the criteria of the CLP regulation, bupirimate should be classified as Skin Sens. 1B as >30% of the animals tested in a Magnusson & Kligman sensitisation study showed a positive response after induction with a 10% concentration. It is therefore proposed to add bupirimate to CLP Annex VI, part 3, Table 3.1.

Bupirimate is not rapidly degradable. It is proposed to classify bupririmate as Aquatic Chronic 1 (H410) based on a NOEC value of 0.10 mg/L in fish. A harmonized M-factor of 1 for chronic toxicity in accordance with the 2nd ATP criteria is proposed.

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	Not classified	none	Not classified	conclusive but not sufficient for classification
2.2.	Flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.3.	Flammable aerosols	Not classified	none	Not classified	conclusive but not sufficient for classification
2.4.	Oxidising gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.5.	Gases under pressure	Not classified	none	Not classified	conclusive but not sufficient for classification
2.6.	Flammable liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.7.	Flammable solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	Not classified	none	Not classified	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	Not classified	none	Not classified	conclusive but not sufficient for classification
2.13.	Oxidising liquids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.14.	Oxidising solids	Not classified	none	Not classified	conclusive but not sufficient for classification
2.15.	Organic peroxides	Not classified	none	Not classified	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	Not classified	none	Not classified	conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	Not classified	none	Not classified	conclusive but not sufficient for classification

	Acute toxicity - dermal	Not classified	none	Not classified	
	Acute toxicity - inhalation	Not classified	none	Not classified	conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	Not classified	none	Not classified	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Not classified	none	Not classified	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	Not classified	none	Not classified	no data
3.4.	Skin sensitisation	Skin Sens. 1B, H317	none	Not classified	
3.5.	Germ cell mutagenicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.6.	Carcinogenicity	Carc 2 (H351)	none	Not classified	
3.7.	Reproductive toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
3.10.	Aspiration hazard	Not classified	none	Not classified	conclusive but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 1, H410	Chronic M-factor 1	Not classified	
5.1.	Hazardous to the ozone layer	Not classified	none	Not classified	conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning

Pictogram: GHS07, GHS09

Hazard statements: H351; Suspected of causing cancer
H317; May cause an allergic skin reaction,
H410; Very toxic to aquatic life with long lasting effects

Precautionary statements: No precautionary statements are proposed since precautionary statements are not included in Annex VI of Regulation EC no. 1272/2008.

Proposed notes assigned to an entry:

A note is not proposed.

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	Not classified	none	Not classified	conclusive but not sufficient for classification
Oxidising properties	Not classified	none	Not classified	conclusive but not sufficient for classification
Flammability	Not classified	none	Not classified	conclusive but not sufficient for classification
Other physico-chemical properties	Not classified	none	Not classified	conclusive but not sufficient for classification
Thermal stability	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	Not classified	none	Not classified	conclusive but not sufficient for classification
Repeated dose toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Irritation / Corrosion	Not classified	none	Not classified	conclusive but not sufficient for classification
Sensitisation	Xi, R43	none	Not classified	
Carcinogenicity	Xn, R40	none	Not classified	
Mutagenicity – Genetic toxicity	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – development	Not classified	none	Not classified	conclusive but not sufficient for classification
Toxicity to reproduction – breastfed babies. Effects on or via lactation	Not classified	none	Not classified	conclusive but not sufficient for classification
Environment	N, R51/R53	none	Not classified	

[1)] Including SCLs ,[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Indication of danger: Xn; N: Harmful; Dangerous for the environment

R-phrases: R40; Limited evidence of a carcinogenic effect
R43: May cause sensitisation by skin contact
R51/53: Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

S-phrases: S36/S37: Wear suitable protective clothing and gloves,
S61: Avoid release to the environment. Refer to special instructions/safety data sheet

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Bupirimate has been assessed in the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of bupirimate in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, March 2007 and subsequent addenda (2009 and 2010, RMS the Netherlands) concerning the placing of plant protection products on the market.

The conclusions on the peer review of pesticide risk assessment of bupirimate were published in the EFSA Journal (8(10):1786, 2010). EFSA proposed the following classification with regard to mammalian toxicological data Xi;R43 "may cause sensitization by skin contact" and with regard to ecotoxicological data N; R51/R53 "Toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment".

The DAR is available via: http://dar.efsa.europa.eu/dar-web/provision

2.2 Short summary of the scientific justification for the CLH proposal

Buprimate induced a small but statistically significant increase in skin fibroma in female rats that was outside the range of the historical controls. In males there was a comparable increase though not statistically significant and within the historical controls. There is no information on the mechanism. Therefore it has to be assumed that this type of tumour is relevant to humans. Classification as Carc. 2 (H351) and Carc Cat 3; R40 is proposed because the carcinogenic effect was only observed in one species and bupirimate is not genotoxic.

A weight of evidence evaluation is required for the skin sensitising properties of bupirimate as the substance is positive in a maximisation test, negative in an LLNA and there is one human case. The human case confirms the positive result in the maximisation test, therefore classification with Skin Sens. 1B; H317 is proposed.

Bupirimate is not rapidly degradable. It is proposed to classify bupirimate as Aquatic Chronic 1 (H410) based on a NOEC of 0.10 mg/L in fish. A harmonized chronic M-factor of 1 in accordance with the 2nd ATP criteria is proposed.

2.3 Current harmonised classification and labelling

Current classification and labelling in Annex VI, Table 3.1 in the CLP Regulation

No harmonised classification exists for bupirimate in Annex VI, table 3.1.

2.3.1 Current classification and labelling in Annex VI, Table 3.2 in the CLP Regulation

No harmonised classification exists for bupirimate in Annex VI, table 3.2.

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Notified classification and labelling according to CLP criteria in CnL Inventory (version 31/05/2012)

Classification		Labelling			Specific Concentration limits, M-Factors	Notes	Number of Notifiers	Joint Entries	View
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms Signal Word Code(s)					
Acute Tox. 3	H311	H311		GHS06 Dgr			23		
Skin Sens. 1	H317	H317		GHS07 GHS09 Wng			20		
Aquatic Chronic 2	H411	H411							

2.4.2 Current self-classification and labelling based on DSD criteria

No information available on self-classification based on DSD criteria for bupirimate.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Bupirimate is an active substance in the meaning of Directive 91/414/EEC and according to article 36 of CLP such substances are subject to harmonised classification.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

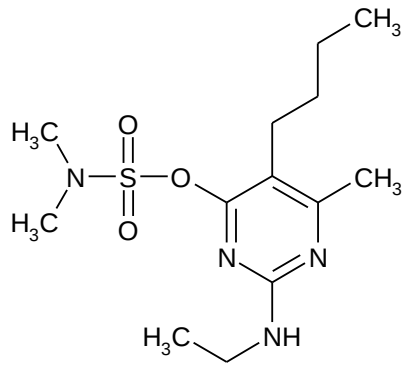
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	255-391-2
EC name:	5-butyl-2-ethylamino-6-methylpyrimidin-4-yl dimethylsulphamate
CAS number (EC inventory):	
CAS number:	41483-43-6
CAS name:	Sulfamic acid, N,N-dimethyl-, 5-butyl-2--(ethylamino)-6-methyl-4-pyrimidinyl ester
IUPAC name:	5-butyl-2-ethylamino-6-methylpyrimidine-4-yl dimethylsulfamate
CLP Annex VI Index number:	
ISO name	bupirimate
Molecular formula:	$C_{13}H_{24}N_4O_3S$
Molecular weight range:	316.4

Structural formula:



1.2 Composition of the substance

The minimum content is 945 g/kg bupirimate in the manufactured material (technical a.i.), on a dry weight basis.

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Bupirimate	≥ 94.5% (w/w)	94.5%-98.2%	

Current Annex VI entry: Not applicable

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Toluene	≤0.3%		The concentration of toluene does not influence the classification of bupirimate
Ethirimol	≤0.2%		The concentration of ethirimol does not influence the classification of bupirimate

Current Annex VI entry:

Toluene (index number 601-021-00-3)
Table 3.1: Flam. Liq. 2 (H225), Repr. 2 (H361d***), Asp. Tox. 1 (H304), STOT RE 2 * (H373**), Skin Irrit. 2 (H315), STOT SE 3 (H336)
Table 3.2: F; R11, Repr. Cat. 3; R63, Xn; R48/20-65, Xi; R38, R67

Ethirimol (index number 603-086-00-3)

Table 3.1: Acute Tox 4* (H312)
Table 3.2: Xn; R21

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
				The substance does not contain additives relevant for harmonised classification and labelling

Current Annex VI entry: Not applicable

1.2.1 Composition of test material

The human toxicity studies were performed with older batches for which only information on purity is available, not on impurities. However, taking into account the used purity of 90-100% in the toxicological studies, the proposed specification of ca 95%, the observed effects, the statement that the manufacturing techniques are similar to the ones used at the time of the studies, it can be concluded that the batches in the studies are comparable to the manufacturing specification, and that the results from the animal studies can be used.

1.3 Physico-chemical properties

Table 9: Summary of physico - chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101.3 kPa	Pure: fine powder free from visible extraneous matter	DAR B.2.1.7. (IIA 2.4) (Bernes, 2002a)	Appearance physical state, visual observation
Melting/freezing point	Mean 44.5-49.3°C (n=2)	DAR B.2.1.1. (IIA 2.1) (Bernes, 2002a)	EEC A1, capillary method, melting temperature device with liquid bath (pure 98.2%)
Boiling point	Mean 232°C (n=2) at 100.13 kPa	DAR B.2.1.2. (IIA 2.1) (Bernes, 2002a)	EEC A2 method according to Siwoloboff, modified principle (pure 98.2%).
Relative density	At 20 ± 0.5°C: mean D^{20}_4= 1.1890 (n=2)	DAR B.2.1.4. (IIA 2.2) (Bernes, 2002a)	EEC A3 method not specified. Calculated against a published value at 4°C: 0.999973 g/ml (pure 98.2%)
Vapour pressure	At 49.2°C: mean 4.58 x 10^{-3}Pa (n=3), melt At 39.4°C: mean 1.64 x 10^{-3}Pa (n=3), solid At 33.5°C: mean 4.19 x 10^{-4}Pa (n=3), solid At 25°C: extrapolated to 1.3 x 10^{-4}Pa At 20°C: extrapolated to 0.57 x 10^{-4}Pa	DAR B.2.1.5. (IIA 2.3) (Mak, 2002b)	EEC A4 gas saturation method (pure 98.2%). Extrapolation reproducibility of the measured values corresponds with those mentioned in EEC A4. The extrapolations are therefore accepted
Surface tension	35.4 mN/m (n=6) at 25ºC (90% solution). Bupirimate is considered surface-active.	DAR B.2.1.24. (IIA 2.14) (Bernes, 2002a)	EEC A5 tensiometer with plate of wilhelmy
Water solubility	Solubility at 20°± 0.5C, at pH 7: 13.06 mg/L (n=40)	DAR B.2.1.11. (IIA 2.6) (Bernes, 2002a)	EEC A6 column elution method
Partition coefficient n-octanol/water	at 21 ± 1°C, at pH 6.92: log Kow=3.68 ± 0.22 (n=6)	DAR B.2.1.13. (IIA 2.8) (Bernes, 2002a)	EEC A8 shake-flask method (pure 98.2%)
Flash point	Not required because melting point is above 40 °C	DAR B.2.1.21. (IIA 2.12) (document MII)	
Flammability	The test item was ignited but the flame did not propagate. Not highly flammable	DAR B.2.1.13. (IIA 2.8) (Bernes, 2002a)	EEC A10 (93.6% pure)
Explosive properties	No explosive properties: test substance does not explode under the effect of a flame, is not sensitive to shock or	DAR B.2.1.21 (IIA 2.13) (Mak, 2002a)	EECA14 (93.6% pure)

	friction		
Self-ignition temperature	No relative self-ignition temperature. Not auto-flammable	DAR B.2.1.20 (IIA 2.11) (Mak, 2002a)	EEC A16 (93.6% pure)
Oxidising properties	Considering the molecular structure and mass and the composition of the material, oxidizing properties are not expected	DAR B.2.1.23 (IIA 2.15) (Mak, 2002a, 2002b)	Evaluation on theoretical basis
Granulometry	Not determined		
Stability in organic solvents and identity of relevant degradation products	Not determined		
Dissociation constant	A pKa of 4.4 is found for the equilibrium of Bupirimate-H^+ + H_2O↔ Bupirimate + H_3O^+	DAR B.2.1.18 (IIA 2.9) (Mak 2002b)	Capillary electrophoresis (CE) (98.2% pure)

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for this dossier.

2.2 Identified uses

Bupirimate is used as fungicide in agriculture, horticulture, pest controlled and protected crops.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

The physico-chemical properties of bupirimate were assessed in the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of bupirimate in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, March 2007 and subsequent addenda (2009 and 2010, RMS the Netherlands) concerning the placing of plant protection products on the market.

Bupirimate has no explosive properties as shown in the EEC A14 test, is a solid that can be ignited but does not propagate (EEC A10) and the molecular structure, mass and composition do not indicate oxidizing properties. Therefore, no classification of bupirimate for physico-chemical properties is required.

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.

4 HUMAN HEALTH HAZARD ASSESSMENT

The human health hazards of bupirimate were assessed in the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of bupirimate in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, November 2009 concerning the placing of plant protection products on the market.

The summaries included in this proposal are copied from the DAR (and its addenda and assessment reports when these contain updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarized in a table. Detailed information is only included for the key study used to derive the classification. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda.

After finalisation of the EFSA evaluation, an LLNA with bupirimate was provided by the notifier to the Netherlands and included in this proposal (Wieland, 2012).

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

See summary below.

4.1.2 Human information

4.1.3 Summary and discussion on toxicokinetics

In rats, ^{14}C-bupirimate is rapidly absorbed and metabolised after oral intake, as most of the bupirimate equivalent is excreted in the urine within 24 hours after intake (64-68%), without the parent compound being detected in the urine (DAR: Prout, 1977b; Mills and Jones, 1974). Within 48 hours, 93% of bupirimate equivalent is eliminated (71-75% by urine, and 18-22% by faeces) (DAR: Prout, 1977b; Mills and Jones, 1974). As 48% of the dose was excreted in the bile within 24 hours, enterophatic circulation occurs and a proportion of faecal metabolites will be of biliary origin.

Results of metabolism studies with orally administrated bupirimate to rats suggest that the initial step in the metabolism pathway of bupirimate is the loss of the dimethylsulphamate side chain, giving rise to a metabolite known as ethirimol (5-butyl-2-ethylamino-4-hydroxy-6-methyl pyrimidine) (DAR: Prout, 1977b). This is supported by an *in vitro* study, in which after 2 hours of incubation of rat liver fractions with bupirimate, the main metabolite identified was ethirimol and a second metabolite was de-ethylated-ethirimol (DAR: Haynes, 2004).

After oral dosing with either bupirimate (rats), or ethirimol (rats and dogs), no bupirimate and no, or only low amounts (≤ 3%) of ethirimol are found in urine, showing rapid metabolism of both bupirimate and its primary breakdown product ethirimol.Ethirimol is further metabolised by N-dealkylation, hydroxylation of the butyl substituent and glucuronidation. The major metabolite in urine after administration of bupirimate, and at the same time one of the major metabolites after administration of ethirimol, is 3-hydroxy-ethirimol (4-(2-hydroxy-4-ethylamino-6-methyl-pyrimidinyl)-butan-2-ol). Furthermore, de-ethylated-ethirimol, 3-hydroxy- de-ethylated-ethirimol and 3-hydroxy-de-ethylated-N-methyl-ethirimol were major metabolites in urine after ethirimol dosing. Ethirimol-glucuronide was the main metabolite in bile. Other metabolites identified in urine and/or bile are ethyl-guanidine and 2-hydroxy-de-ethylated-ethirimol (1-(2-hydroxy-4-amino-6-methylpyrimidinyl)butan-2-ol). (DAR: Prout, 1977b; Bratt et al., 1972).

Bupirimate (rats) or ethirimol or their metabolites do not seem to accumulate in the fat and tissue retention is low following repeated oral dosing. The excretion rate of radioactivity in faeces and urine seemed to be biphasic with an initial $t_{½}$ of 7-12 hours and a second $t_{½}$ of approximately 36 hours after administration of bupirimate, or 75 hours after administration of ethirimol (DAR: Mills and Jones, 1974). The highest concentration of equivalents in tissues is found in the liver, which is 0.3% of the administrated dose after 20-28 days of dosing of either ethirimol or bupirimate (DAR; Mills and Jones, 1974. No major metabolic differences were found between rats and dogs.

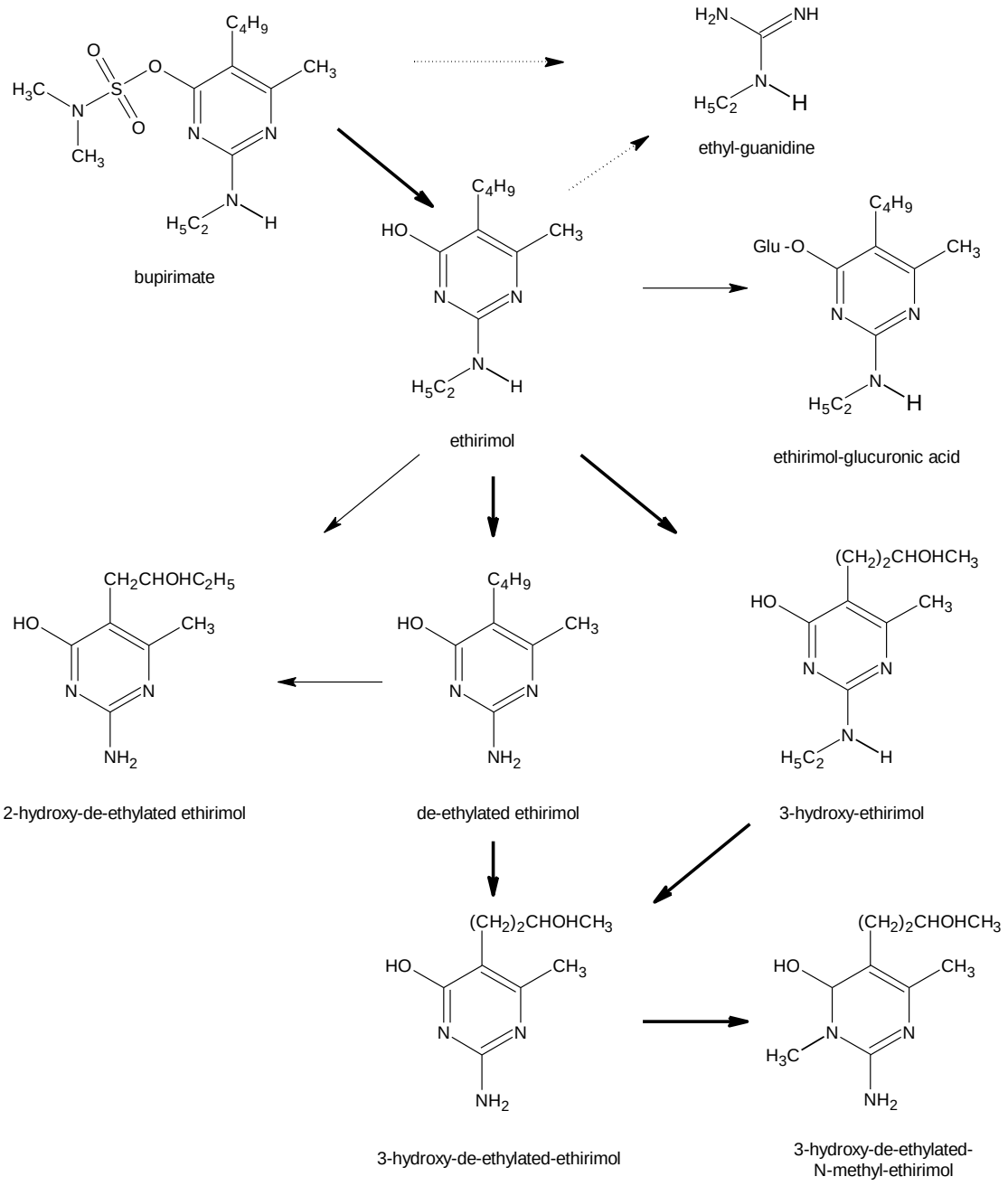
As bupirimate was readily absorbed, oral absorption is assumed to be 100%.

Two *in vitro* dermal absorption studies have been conducted, both using rat and human skin membranes. In both studies the same dose levels were used, i.e. undiluted formulation (high dose, corresponding to exposure during mixing and loading) and the highest and lowest spray-dilutions for field use (mid and low dose). In the first study (DAR: Maas, 2002) the penetration of bupirimate through human epidermis during 24 hours continuous exposure was 13%, 15% and 1.3% for low,

mid and high dose, respectively. Bupirimate present in the skin should be added to those values to calculate the maximum absorption percentage, as bupirimate in the skin can at a latter stage also penetrate the skin. Absorption is than 42% for the concentrate and maximum 58% for the spray dilution. The membrane wash at the end of the exposure period removed 38-54% of the radioactivity from the human epidermal membranes.

In the second study (DAR: Maas, 2004), bupirimate was formulated as NIMROD 25 EC and NIMROD 25 EW and skin was washed after an 8 hour exposure period and absorption measured for a further 16 hour period. Total absorption (i.e. penetrated bupirimate plus bupirimate present in desquamated skin) of the NIMROD 25 EC formulation through human skin was 11.6%, 7.9% and 1.3% following application of low, mid and high dose, respectively. These results are similar to that obtained with NIMROD 25 EW. Tape stripping experiments showed that radioactivity remaining in skin 24 hours after application was present in the outer layers and therefore would be lost by desquamation. Absorption through rat skin was generally slightly higher; in both studies the flux constants measured on the linear part of the absorption-time curve were 2 to 8 times higher for rat skin than for human skin.

The second study (DAR: Maas, 2004) provides more realistic data for use in the exposure assessment. Based on the human *in vitro* data absorption of the concentrate is 1.3% and absorption of bupirimate from spray dilutions is maximum 12%.


bupirimate
ethyl-guanidine
ethirimol
ethirimol-glucuronic acid
2-hydroxy-de-ethylated ethirimol
de-ethylated ethirimol
3-hydroxy-ethirimol
3-hydroxy-de-ethylated-ethirimol
3-hydroxy-de-ethylated-
N-methyl-ethirimol

4.2 Acute toxicity

Table 10: Summary table of relevant acute toxicity studies

Method	Test details	Results (LD_{50}/LC_{50})	Study quality (Klimisch score)	Reference
Oral Toxicity				
Acute LD_{50} Resembling OECD 401, No GLP	Rat, 6f/group No control mentioned Dose levels: 1000, 2000, 2500, 3200 and 4000 mg/kg bw	Approx. 4000 mg/kg bw	Reliable with restrictions	DAR (Gore et al, 1975)
Acute LD_{50}, limit test Resembling OECD 401, No GLP	Rat, 5/sex/group No control mentioned Dose: 4000 mg/kg bw	> 4000 mg/kg bw	Reliable with restrictions	DAR (Henderson, 1981)
Acute LD_{50} Resembling OECD 401, No GLP	Mouse, 6f/group No control mentioned Dose: up to 4000 mg/kg bw	> 4000 mg/kg bw	Reliable with restrictions	DAR (Gore et al, 1975)
Acute LD_{50} Resembling OECD 401, No GLP	Guinea pig (Dunkin-Hartley), 4m/group No control mentioned Dose: 2000 and 4000 mg/kg bw	Approx. 4000 mg/kg bw	Reliable with restrictions	DAR (Gore et al, 1975)
Acute LD_{50} Resembling OECD 401, No GLP	Rabbit (NZW), 2f/group No control mentioned Dose: 1000, 2000 and 4000 mg/kg bw	2000-4000 mg/kg bw	Reliable with restrictions	DAR (Gore et al, 1975)
Inhalation Toxicity				
Acute LC_{50}, limit test No guideline No GLP	Rat, 2/sex/group No control mentioned Dose: 35 mg/m^3 4 h exposure	>35 mg/m^3	Not reliable	DAR (Carney, 1976)
Acute LC_{50} limit test OECD 403 GLP	Rat, 5/sex/group Nominal dose: 1.62 mg/l, actual dose: 1.34 mg/l 4 h exposure	>1.34 mg/L	Reliable without restrictions	DAR addendum 2010 (Kaniuk, 2003)

Dermal Toxicity				
Acute LD50, limit test No guideline No GLP	Rat, 3f/group No control mentioned Dose of 4 mL/kg bw (conc. not specified) 24 h exposure	No mortalities	Not reliable	DAR, Gore *et al.* (1975)
Before OECD Guidance, but resembling OECD 402 limit test No GLP	Rabbit, 4/sex/group No control mentioned Dose: 2000 mg/kg bw 24 h exposure	2000 mg/kg bw	Reliable with restrictions	DAR (Henderson, 1981)
Intraperitoneal Toxicity				
No guideline No GLP	Rat, 3/sex/group No control mentioned Dose: 1600, 2000 and 4000 mL/kg bw	2000-4000 mg/kg bw	Reliable with restrictions	DAR (Gore et al., 1975)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Rats

Bupirimate was administered either in aqueous suspension or propylene glycol solution (not specified which used; DAR: Gore *et al*., 1975). Groups of six females were treated once orally, by gavages, at dose levels of 1000, 2000, 2500, 3200 and 4000 mg/kg bw, following an overnight fast. Animals were observed for 14 days after administration. No necropsy was conducted. The acute oral media lethal dose (LD_{50}) was calculated using a moving average interpolation technique. The highest dose level at which deaths did not occur was 3200 mg/kg. there were two deaths at 4000 mg/kg; one in the first 24 hours, and a second on day 2. Clinical signs prior to death were not reported. The remaining animals survived until day 14. All rats (dose level 1000 to 4000 mg/kg) had signs of incontinence after 18/24 hours. Some animals at the higher doses were subdued (dose levels not defined). Surviving animals had fully recovered by day 7. The following LD_{50} was established: LD_{50} ~ 4000 mg/kg bw

Bupirimate, as an aqueous suspension in 0.5% “lissatan” AC, was administered by oral gavages to five male and five female rats (source and weight not specified) at a dose level of 4000 mg/kg bw (DAR: Henderson, 1981). The animals were fasted for 16-20 hours prior to administration and observed for 14 days. No deaths occurred following administration of 4000 mg/kg bw. Signs of toxicity, apparent three hours after dose administration, were described as subdued behaviour, urinary incontinence and un-groomed appearance. The signs persisted for up to 10 days. The following LD_{50} was established by this limit test: LD_{50} >4000 mg/kg bw

Mouse

Bupirimate was administered either in aqueous suspension or propylene glycol solution (not specified which used; DAR: Gore *et al*., 1975)). Groups of six females mice were treated once orally, by gavages, at dose levels up to 4000 mg/kg bw (lower dose levels not specified), following

an overnight fast. Animals were observed for 14 days after administration. No necropsy was conducted. No mortalities were observed. Some animals became slightly subdued (dose and timing not specified) but had fully recovered within six days. The following LD_{50} was established: LD_{50} >4000 mg/kg bw

Guinea pig

Bupirimate was administered either in aqueous suspension or propylene glycol solution (not specified which used; DAR: Gore *et al*., 1975). Groups of four male guinea pigs were treated once orally, by gavages, at dose levels of 2000 and 4000 mg/kg bw (lower dose levels not specified), following an overnight fast. Animals were observed for 14 days after administration. No necropsy was conducted. The highest dose level at which death did not occur was 2000 mg/kg bw. There were two death at 4000 mg/kg bw, on day 2 and 4. The remaining animals survived until day 14. Toxic signs included an increase in the depth of respiration and slight salivation (dose and timing not specified).The following LD_{50} was established: LD_{50} ~4000 mg/kg bw

Rabbit

Bupirimate was administered either in aqueous suspension or propylene glycol solution (not specified which used; DAR: Gore *et al*., 1975). Groups of two female rabbits were treated once orally, by gavages, at dose levels of 1000, 2000, and 4000 mg/kg bw, following an overnight fast. Animals were observed for 14 days after administration. No necropsy was conducted. The highest dose level at which death did not occur was 2000 mg/kg bw. At 4000 mg/kg bw both rabbits died at 4000 mg/kg bw within the first 24 hours after administration. The remaining animals became subdued (timing not specified), but had fully recovered within three days and survived until day 14. The following LD_{50} was established: LD_{50} 2000-4000 mg/kg bw

4.2.1.2 Acute toxicity: inhalation

The only reliable study was performed in accordance with OECD 403 (DAR: Kaniuk, 2003) . Due to physico chemical characteristics of the test material, the maximum tested nominal concentration was 1.62 mg/l air (actual 1.34 mg/l). Groups of 5 male and 5 female rats were treated. This resulted in no mortality, no symptoms of toxicity and no treatment-related findings regarding their body weight or pathology. The following LD_{50} was established: LD_{50} > 1.34 mg/l for both male and female rats, the highest attainable concentration.

4.2.1.3 Acute toxicity: dermal

In the only reliable study, bupirimate was applied as a 50% (w/w) suspension in propylene glycol, to an area (5 cm x 10 cm) on the clipped backs of a group of four male and four female rabbits (New Zealand White) at a dose level of 2000 mg/kg bw (DAR: Henderson, 1981). Immediately before administration, longitudinal epidermal abrasions were made on the treated area of two males and two females. Contact with the skin was maintained for 24 hours, using an occlusive dressing. The skin was then cleansed with 190 Cetrimide solution and the animals observed for 14 days. No mortalities were observed. No toxic signs were reported. The following acute dermal LD_{50} was established: LD_{50} > 2000 mg/l for both male and female rats.

4.2.1.4 Acute toxicity: other routes

Bupirimate was administered in aqueous suspension by intraperitoneal injection (DAR: Gore *et al*., 1975). Groups of three rats of each sex were treated once at dose levels of 1600, 2000 and 4000 mg/kg bw. Animals were observed for 14 days after the injection. No necropsy was concluded. The highest dose level at which deaths did not occur was 2000 mg/kg bw. At 4000 mg/kg bw, two female rats dies within the first 24 hours after administration, while two male rats died three days after administration. The remaining animals became subdued (timing not specified), but has fully recovered within three days and survived until day 14. The remaining male and female rat survived until day 14. All animals showed urinary incontinence and those on the top two doses were subdued (timing not specified). The survivals appeared normal after 5 days. The following acute intraperitoneal LD_{50} was estimated: LD_{50} 2000-4000 mg/kg bw for both male and female rats.

4.2.2 Human information

4.2.3 Summary and discussion of acute toxicity

Bupirimate has a low order of acute oral, dermal, inhalation and intra-peritoneal toxicity in the rat. The principle signs of acute oral over-dosage in the rat are urinary incontinence, subdued behaviour and an ungroomed appearance. Dermal applied bupirimate at levels not yet lethal to rats caused urinary incontinence in these animals, suggesting dermal exposure was associated with toxic systemic effects. In the acute inhalation study no treatment related effects were observed at 1.34 mg/L, the highest attainable concentration. Acute oral toxicity of bupirimate is also shown to be low in the mouse, rabbit and guinea pig.

4.2.4 Comparison with criteria

Based on the oral acute toxicity tests on rats ($LD_{50}\geq$ 4000 mg/kg bw) and according to the EC classification criteria, bupirimate should not be classified for acute oral toxicity. For acute dermal toxicity, only a limit test on rabbits is available showing no signs of toxicity and mortality at 2000 mg/kg bw. Therefore, no classification for acute dermal toxicity is required according to the EC classification criteria. In the acute inhalation test in rats, no mortality, no symptoms of toxicity and no treatment-related findings regarding their body weight or pathology were observed at the highest attainable concentration of 1.34 mg/l (LD_{50} > 1.34 mg/l).

4.2.5 Conclusion on classification and labelling

No classification for acute toxicity through the oral, dermal and inhalatory route is warranted based on the criteria of the DSD and CLP.

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.

4.3 Specific target organ toxicity – single exposure (STOT-SE)

4.3.1 Summary and discussion of Specific target organ toxicity– single exposure

In the first acute oral toxicity in the rat, all rats (dose level 1000 to 4000 mg/kg) had signs of incontinence after 18/24 hours (DAR: Gore *et al*., 1975). In the second acute oral study, rats were administered 4000 mg/kg (DAR: Henderson, 1981). Signs of toxicity, apparent three hours after dose administration, were described as subdued behaviour, urinary incontinence and un-groomed appearance. The signs persisted for up to 10 days. In the mouse study some animals became slightly subdued (dose and timing not specified) but had fully recovered within six days (DAR: Gore *et al*., 1975). In the acute oral study in guinea-pigs the toxic signs included an increase in the depth of respiration and slight salivation (dose and timing not specified; DAR: Gore *et al*., 1975). In the rabbit study, the remaining animals became subdued (timing not specified), but had fully recovered within three days and survived until day 14 (DAR: Gore *et al*., 1975).

In the acute inhalation study with rats the maximum tested nominal concentration was 1.62 mg/l air (actual 1.34 mg/l) and resulted in no mortality, no symptoms of toxicity and no treatment-related findings regarding their body weight or pathology (DAR: Kaniuk, 2003).

No toxic signs were reported in the acute dermal toxicity study in rabbits at the dose level of 2000 mg/kg bw (DAR: Henderson, 1981).

4.3.2 Comparison with criteria

The effects observed in the available acute toxicity studies via the three different routes do not indicate a specific target organ toxicity after single exposure.

4.3.3 Conclusion on classification and labelling

No classification for irreversible effects after single exposure or STOT-SE through the oral, dermal and inhalatory route is required under DSD and CLP.

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.

4.4 Irritation

Table 11: Summary table of relevant irritation studies

Type of study	Dose level (mg/kg b.w.)	Animal species, sex; strain	Findings	Study Quality (Klimisch score)	References
Skin irritation					
Irritation study-Non guideline, supplementary No GLP	1 ml/kg bw of 25% w/v solution; skin	Rat, 6 f	No skin irritation	Not reliable	DAR (Gore et al., 1975)
Irritation study-EC B.4, OECD 404 GLP	500 mg as paste in water; skin	Rabbit, 3 m	No skin irritation	Reliable without restrictions	DAR (Leuschner et al., 2001a)
Eye irritation					
Irritation study	One drop of a	Rabbit, 2 f, one	Mildly irritating	Not reliable	DAR (Gore et

No guideline No GLP	25% w/v solution	eye dosed/animal	Moderate initial pain (class 3), slight redness and chemosis of the conjunctivae (Draize score 1), some discharge. Effects lasted for 4 days.		al., 1975)
Irritation study-resembling OECD 405 No GLP	100 mg/test-eye	Rabbit, 6 f, one eye dosed/animal	Very slightly irritating Moderate initial pain (class 3), slight redness conjunctivae, slight to mild chemosis of the conjunctivae, some discharge. Normal after three days.	Reliable with restrictions	DAR (Henderson, 1981)
Irritation study-EC B5/OECD 405 GLP	100 mg/test-eye	Rabbit, 3 m, one eye dosed/animal	Non- irritating	Reliable without restrictions	DAR (Leuschner, 2001b)

4.4.1 Skin irritation

Acceptable acute irritation studies were available. Bupirimate showed to be not a skin irritant.

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.

4.4.2 Eye irritation

Acceptable acute irritation studies were available. Bupirimate showed to be very slightly irritating to the eyes, but not severe enough for classification.

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.

4.5 Sensitisation

4.5.1 Skin sensitisation

Table 12: Summary table of relevant skin sensitisation studies

Method	Test details	Results	Study quality (Klimisch score)	Reference
Sensitisation study No guideline No GLP	Guinea pigs, 4 (sex not specified) 3 control animals	No skin sensitisation	Not reliable	DAR (Gore et al., 1975)
"M&K"-method-Sensitisation study-resembles OECD 406 GLP	Guinea pigs; 20 f exposed. Vehicle control with 10 f, positive control with 20 m.	Moderate skin sensitizer	Reliable with retrictions	DAR (Rattray et al., 1990)
Skin Sensitization: Local Lymph Node Assay, No guideline GLP	Mice, 6f/group, vehicle control with 6 f, positive control with 6 f Doses: 10%, 25% and 50%	No skin sensitisation	Not reliable	DAR addendum 2010 (Haferkorn, 2000)
Skin Sensitization: Local Lymph Node Assay, OECD 429 GLP	Mice, 4 f/group, vehicle control with 4 f	No skin sensitisation.	Reliable without restrictions	Wieland, 2012

4.5.1.1 Non-human information

Twenty test animals received three pairs of intradermal injections (0.05-0.1 mL each) in the clipped skin on each side of the spine: 1) Freund's Complete Adjuvant (FCA) + corn oil (1:1), 2) a 10% w/v solution of bupirimate (CTL reference number Y00352/010/002; purity 90.3% w/w) in corn oil, and 3) a 10% w/v solution of bupirimate in a 1:1 mixture of FCA and corn oil (DAR: Rattray *et al*.,

1990). One week after the intradermal injections the animals were exposed to 75% w/v sE (0.2-0.3 mL) by topical application under occlusion for 48h. Ten vehicle control animals (f) were treated identically for both intradermal and topical induction, but with the absence of bupirimate.

Following challenge with 75% w/v bupirimate preparation (0.05-0.1ml in corn oil, right flank, 24 hours under occlusion), mild to intense redness and swelling was seen on 14/20 and 8/20 test animals after 24 and 48 hours respectively, whereas scattered mild redness was seen on 2/10, respectively 0/10 control animals. Following challenge with 30% w/v bupirimate preparation (0.05-0.1 ml in corn oil, left flank, 24 hours under occlusion), scattered mild redness or moderate diffuse redness was seen on 9/20 and 4/20 test animals after 24 and 48 hours respectively. In control rats scattered redness was seen on 1/10 and 0/10 animals after 24 hours and 48 hours, respectively. Body weight gain was similar in all groups.

In the positive control study, a challenge with 30% w/v preparation of a 40% aqueous formaldehyde solution elicited a mild to moderate skin sensitisation response in previously induced male guinea pigs.

This well described study resembles OECD 406 with the following minor deviation on: skin irritation scores after induction were not reported. Furthermore, the challenge with bupirimate should have been carried out with a non-irritating concentration; the results of the sighting study are not available, hence it is unclear why two challenge concentrations were tested. The study protocol indicates that in the sighting study preparations in corn oil were tested to determine a.o. the non-irritating concentration in FCA treated animals for the challenge applications in the main study. Despite this inadequacy, the study was considered acceptable.

The skin sensitizing potential of Bupirimate (purity 95.2%) was also tested by using the local lymph node assay (LLNA; Wieland, 2012). This test was performed according to the OECD Guideline 429. The test animals (mice, strain CBA/CaOlaHsd, females, 8-9 wk, n=4) were treated by topical application on the ear with Bupirimate. The test item (10, 25 and 50%, w/v, in vehicle aceton: olive, 4+1 v/v) was applied on the dorsal surface of each ear once a day for three consecutive days (25 µl/ear/day). The concentration of 50% was the highest technically achievable. The control group of mice was treated with the vehicle alone. Five days after the first application the mice (test and control group) were injected into the tail vein with 250 µl PBS, containing 20.1 µCi ^{3}H-methyl thymidine. Five hours later the mice were sacrificed. The draining lymph nodes were excised and pooled per group. The incorporation ^{3}HTdR was measured by counting the incorporation of ^{3}H-methyl thymidine by ß-scintillation.

No signs of systemic toxicity were observed in the treated animals. On day 3, 4, 5 and 6, all treated animals showed a very slight erythema (score 1) of the ear for all test item concentrations except the 50 % concentration on day 4 and 5 which showed a well defined erythema (score 2). The incorporation of radio-activity of each treatment group, expressed as the SI (simulation index) was below 3 (2.26, 1.84 and 1.52 for 10, 20 and 50% respectively). An EC3 value could not be calculated, since none of the tested concentrations induced a SI above the threshold value of 3. From these results it can be concluded that the test item Bupirimate did not show sensitising properties in the local lymph node assay .

4.5.1.2 Human information

Bupirimate has been manufactured and formulations produced in the UK for the last 25 years. During that period one case of skin sensitisation was documented involving a formulation worker,

this was confirmed by patch testing. Three cases of irritation/sensitisation have been recorded during manufacture, the sensitisations were not confirmed by patch testing.

4.5.1.3 Summary and discussion of skin sensitisation

In a well described maximisation study on guinea pigs with bupirimate, >30% of the animals showed a positive response at an induction dose of 10%. In the LLNA test, none of the tested concentrations induced a SI above the threshold value of 3. In this test, Bupirimate did not show sensitising properties.

In one documented human case, Bupirimate was found to be a moderate skin sensitizer.

4.5.1.4 Comparison with criteria

According to the DSD and CLP classification criteria, bupirimate should be classified as sensitising as more than 30% of the animals in an adjuvant study reacted positive. However, the LLNA with bupirimate was negative as the SI was below 3 for all tested concentrations. The human evidence was limited to one case. The human evidence is in itself not sufficient to conclude that bupirimate should be considered a skin sensitiser as there is only one case whereas positive data from normally more than one clinic is required for case studies.

The two animal tests have a conflicting result requiring a weight of evidence approach. Both studies are regarded as acceptable studies. Further, the reason for the difference between the two studies remains unknown. Therefore, there is no reason to base the classification on only one of these two animal studies. However, as there is also one human case which confirms that bupirimate can induce skin sensitisation, classification as a skin sensitiser is proposed. According to CLP, a substance should be classified as skin sensitiser, sub-category 1B; H317 when more than 30% of the animals in an adjuvant study reacts positive after induction with a dose of >1%, or when more than 30% but less than 60% reacts positive after induction with >0.1 to ≤1%. A substance should be classified as skin sensitiser, sub-category 1A; H317 when more than 30% of the animals in an adjuvant study reacts positive after induction with a dose of ≤ 0.1%, or more than 60% reacts positive after induction with >0.1 to ≤1%. After an induction dose of 10%, >30% of the animals reacted positive, and therefore classification (at least cat 1B) is required. Since there are no data with induction concentrations of <1% it is not known whether bupirimate would fulfil the requirements of subcategory 1A, based on the maximisation study on guinea pigs. However, because of the negative outcome of the LLNA test at dose > 1%, the sub-category 1A does not appear to be applicable and thus subclassification as skin sensitisation Cat 1B; H317 is proposed.

4.5.1.5 Conclusions on classification and labelling

Table 13 Conclusion on classification for skin sensitisation

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP	Skin Sens. 1B (H317)	Xi: R43

Regulation)		

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%
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
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.

4.5.2 Respiratory sensitisation

4.5.2.1 Non-human information

This information is not available.

4.5.2.2 Human information

No human information is available.

4.5.2.3 Summary and discussion of respiratory sensitisation

This information is not available.

4.5.2.4 Conclusion on classification and labelling

No classification is required due to absence of data.

4.6 Repeated dose toxicity

Table 14: Summary table of relevant repeated dose toxicity studies

Method	Test details	Results (NOAEL/LOAEL)	Study Quality (Klimisch score)	Reference
10-days oral gavage, rat No guideline No GLP	Exposure 1: 10/sex /group Dose: 0, 250, 1000 mg/kg bw/d Exposure 2: 20 f, vehicle control with 10 f Dose: 1000 mg/kg bw	NOAEL < 250 mg/kg bw/day, LOAEL = 250 mg/kg bw/day Urinary incontinence (females), liver weight ↑, kidney weight ↑ (females), creatine clearance ↓ (females), plasma creatine ↑, protein and glucose in urine ↑	Reliable with restrictions	DAR (Ferguson, 1977)
14-day oral gavage, rat No guideline No GLP	10/sex/ group, no control used Dose: 250 and 1000 mg/kg bw/d Dosing: 5 days/week	Some effects at 1000 mg/kg bw/d	Not reliable	DAR (Gore et al., 1975)
14-day dermal, rabbit No guideline No GLP	6 females, no control used Dose: 500 mg/kg bw Dosing: 5 days/week, 18 h/d	Erythema, reduced weight gain, blood urea ↑, moderate kidney damage, mild liver inflammation	Not reliable	DAR (Gore et al., 1975)
90-day oral diet, rat Resembles OECD 408 No GLP	20 /sex /group Dose: 0, 100, 500, 1000, 20000 ppm	NOAEL = 50 mg/kg bw/day (1000 ppm); LOAEL = 1700 mg/kg bw/day (20000 ppm) Body weight gain ↓, liver weight ↑ (females), liver and thyroid weight ↑ (females), platelet count ↓, ASAT ↑ (males),	Reliable with restrictions	DAR (Wheldon et al., 1974a)
90-day oral gavage, dog Resembles OECD 409 No GLP	4/sex/group Dose: 0, 3, 15, 30 and 600 mg/kg bw/day	NOAEL = 15 mg/kg bw/day; LOAEL = 30 mg/kg bw/day Thymus (weight ↓, histopathology), thyroid weight ↑	Reliable with restrictions	DAR (Wheldon et al., 1974b)
104 weeks oral diet, rat Resembled OECD 453	40/sex/group Dose: 0, 100, 1000 and 5000 ppm	NOAEL = 25 mg/kg bw/day (1000 ppm) LOAEL = 156 mg/kg	Reliable with restrictions	DAR (Ben-Dyke et al., 1977)

No GLP		bw/day (5000 ppm) Severly reduced bw		
80 weeks oral diet, mouse No guideline No GLP	40 /sex / group Dose: 0, 100 and 1000 ppm	No evidence of carcinogenicity	Not reliable	DAR (Ben-Dyke et al., 1977b)
105 weeks oral gavage, dog Resembled OECD 452 No GLP	4/sex/group Dose: 0, 5, 20 and 200 mg/kg bw/d	NOAEL = 5 mg/kg bw/day LOAEL = 20 mg/kg bw/day Liver weight ↑ with associated clinical chemistry and histopathology (e.g. pigment-laden macrophages ↑, pigment containing Kupffer cells ↑)	Reliable with restrictions	DAR (Ben-Dyke et al., 1977c)

4.6.1 Non-human information

4.6.1.1 Repeated dose toxicity: oral

In the sub-acute toxicity study with rats (DAR: Ferguson, 1977), in the 1st experiment, ten daily doses (5 days/week, 2 weeks) of bupirimate (purity not specified) were administered in propylene glycol to groups of 10 male and 10 female rats (SPF, albino) by oral gavage at dose levels of 0, 250 and 1000 mg/kg bw/day. Clinical observations and body weight recordings were made daily. Following administration of the last dose, 18-hour urine samples were collected from 5 male and 5 female rats of each group and blood was collected from all animals. Urine samples were analysed for volume, osmolarity, creatine, protein and glucose, blood samples were analysed for biochemical analyses (urea, plasma osmolarity and plasma creatine). All test animals and half of the control animals were killed, and the following tissues removed at necropsy: liver, kidney, adrenal, urinary bladder, lung and heart. Liver and kidney were weighed and examined microscopically.

In the 2nd experiment, a further 30 females were used to assess the reversibility of possible renal dysfunction. 20 female rats received 10 daily doses (5 days/week, 2 weeks0 of 1000 mg/kg bw/day bupirimate in propylene glycol, and 10 control females received the vehicle alone; following administration of the last dose, 18-hour urine samples were collected from 10 treated and 5 control rats; blood samples were taken from all animals. 14 days after the last dose, 18-hour urine samples and blood samples were again collected. The animals were killed and liver and kidney weights were recorded. Blood and urine were analysed for the same parameters, except for blood urea nitrogen. Some group mean data was assessed using the Students "t" test and urinary excretion rats and renal clearance values were calculated.

The treated animals gained the expected bodyweight and appeared in good condition (see table 15). Two females of the 2nd experiment (1000 mg/kg bw/day) died (day 9 and 10) according to the author as a result of misdosing. Urinary incontinence was observed in females in both dosing groups, but only in 1/10 males in the high dose group; the incontinence ceased on cessation of dosing. Plasma creating was increased in male and female rats at both dose levels, plasma urea was significantly decreased in female rats at 1000 mg/kg bw/day. In females, increases in glucose and

protein concentration in urine were found at both dose levels. The creatine clearance was dose-relatedly decreased in females, but returned to normal after the recovery period. Treatment-related and statistically significant increases in absolute and relative weight of liver (both sexes) and kidney(female) were apparent at both dose levels. At the end of the recovery period kidney weights were similar to control values, but liver weights remained slightly elevated. Histopathological examination of kidney and liver revealed no specific abnormalities.

Table 15. Results of a 10-day oral toxicity study in rats (DAR: Ferguson, 1977)

Dose (mg/kg bw/d)	0		250		1000			1000+ Rec.	dr
sex	m	f	m	f	m	f exp.1	f exp. 2	f	
Mortality	no toxicologically relevant effect								
Clinical signs urinary incontinence ungroomed appearance subdued	 0/10 0/10 0/10	 0/10 0/10 0/10	 0/10 0/10 0/10	 6/10 0/10 1/10	 1/10 0/10 0/10	 10/10 5/10 0/10	 16/20 0/20 0/20	 #	 dr R
Body weight gain	no toxicologically relevant effect								
Blood chemistry urea creatine			 is	 is	 is	 ds -	 n.a. is	 n.a. -	 dr R
Urinalysis glucose protein creatine clearance			 i i	 i i d	 i d	 i i d	 i i d	 i i	 dr dr f dr f R
Organ weights (% of control-100)) liver rel. liver kidney rel. kidney			 +16** +21** -10 -8	 +22** * +23** * +10 +10*	 +36*** +52*** -6 +5	 +52*** +57*** +19* +23**	 n.a n.a n.a n.a	 +9* +15*** -2 +4	 dr dr dr f R dr f R
Pathology	no toxicologically relevant effect								

1000+Rec = animals dosed 1000 mg/kg bw/d for 10 days, then were able to recover for 14 days
i = increased; d = decreased; is = increased significantly; ds = decreased significantly
dr = dose related; dr R= dose related but reversible; dr f = dose related in females,
# ceased on cessation of dose administration, n.a. = not analysed, - = no effect measured
*p<0.05, **p<0.01, ***p<0.001 : Student's t test

On the basis of effects on kidneys in females and liver weights in males and females at 250 mg/kg bw/day a NOAEL could not be derived. The LOAEL was 250 mg/kg bw/day.

In another study (Gore, 1975), 10 daily doses (5 days/week, 2 weeks) of bupirimate (purity not speicifed) were administered in aqueous suspension (20% w/v "Dispersol"OG) to groups of 10 male and 10 female rats (SPF albino, 140-220 g) by oral gavage at dose levels of 250 and 1000 mg/kg bw/day. No control group was used. Body weights were measured at the beginning and end of the study. On the day of the last dose administration 18-hour urine samples were collected from 5 male and 5 female rats at each dose level. Blood for haematological (Hb, PVC, MCHC, mean corpuscular diameter or red cells (MCD), RBC, WBC, WBC/diff, platelets, and clothing time) and biochemical (urea, sodium and potassium only) analysis were also collected (route not specified) from these animals. Five males and 5 females from each group were killed (method not specified) and the following tissues taken: liver, kidney, adrenal, spleen, lung, thymus, heart, stomach, duodenum, jejunum, ileum, pancreas, salivary gland, urinary bladder and gonads (including epididymus). Seven days after administration of the last dose the above tissues were taken from a further 3 males and 3 females in each group. Control biochemical blood analysis data from 3 males and 3 females and control urinalysis ddata from 5 males and 5 females from another experiment were presented for comparison.

There were no deaths during the study. Urinary incontinence was noted particularly in females in both dose groups and only in 1/10 males at 1000 mg/kg bw/day. In addition, females at 1000 mg/kg bw/day appeared ungroomed. Body weight gains were statistically significant lower in males at 1000 mg/kg bw/day than in males at 250 mg/kg bw/day. Histopathology showed evidence for mild renal tubuar damage and a mild inflammatory response in the liver in some animals. With lack of a control group, the relevance of these histopathological findings is not known.

As no control group was used and no organ weights were taken, the study is considered acceptable as a range finding study.

In a semi-chronic oral toxicity study (Wheldon, 1974a), bupirimate (purity 99.5%) was given by dietary admixture at concentrations of 0, 100, 500, 1000 and 20000 ppm to 5 groups of 20 male and 20 female Sprague-Dawley rats for 90 days. Clinical observations were recorded daily and body weight and food consumption were measured, as a minimum, weekly. Ophthalmological examinations were made before the start of treatment and in weeks 6 and 13. Blood samples for haematological evaluation (Hb, PCV, MCHC, MCV, RBC, WBC, WBC/diff, platelets and coagulation) were taken from 10 males and 10 females from each group before the start of treatment and in weeks 4 and 12. Blood and urine samples for biochemical examination were taken from 4 males and 4 females from each group before the start of treatment and in weeks 4 and 12. At the end of the treatment all animals were killed and subjected to necropsy. Organ weights were recorded for all animals of 1000 and 20000 ppm groups and from 10 males and 10 females in each other group, and a selection of tissues from all animals was examined microscopically (only results of the control and the group receiving 20,000 ppm were reported).

The average daily intakes were calculated as:

100 ppm: 4.2-16 mg/kg bw/day (males) and 8.3-14 mg/kg bw/day (females)

500 ppm: 24-73 mg/kg bw/day (males) and 33-74 mg/kg bw/day (females)

1000 ppm: 49-157 mg/kg bw/day (males) and 71-129 mg/kg bw/day (females)

20000 ppm: 1698-2523 mg/kg bw/day (males) and 2270-2574 mg/kg bw/day (females)

There were no treatment-related mortalities. In animals of 20000 ppm group, a lack of grooming was apparent from weeks 6 onwards. A treatment-related decrease in body weight gain, which was apparent from the first week of treatment, was seen in both sexes receiving 20000 ppm. In males of 20000 ppm group food consumption was decreased for the first 4 weeks of treatment, but total food consumption over the whole treatment period of all groups was similar to controls. Platelet count decreased with time, but this decrease was dose-relatedly less in treated females. In males, the level of ASAT was significantly increased in the 20000 ppm group. Protein concentrations measured in week 13 were generally lower in urine samples of treated rats, but the response was only weakly dose-related.

There were no treatment-related macroscopic observations at necropsy (table 16). In the highest dose group, absolute organ weights of heart, kidney and pituitary were decreased in male and female; and of lung only in male rats, and liver and thyroid were increased in females (tables 16 and 17). Body weight-related organ weights were increased for brain, gonads, thyroids and liver, and only in male also in adrenals. These changes were all considered a consequence of the lower body weights, except for increased liver and thyroid weights, as both relative and absolute weight of these organs were significantly increase, though only in females. No treatment-related histopathological changes were found.

Table 16. Results of a 90-day oral toxicity study in rats (DAR addendum 2010: Wheldon, 1974a)

Dose (ppm) / Sex	0		100		500		1000		20,000		dr
	m	f	m	f	m	f	m	f	m	f	
Mortality	no toxicologically relevant effect										
Clinical signs ungroomed app.									i	i	
body weight gain % of control -100			-1	5	-3	6	-3	4	-54***	-35***	
Food consumption									d (t)	-	
Urinalysis protein conc. wk 13			ds*	d	d	ds*	ds**	d	ds***	ds**	
Haematology platelets (fraction[a])		0.57	-	0.60	-	0.63	-	0.66	-	0.70	dr f
Clinical Chemistry ASAT							i		is*	-	dr m
Ophtalmoscopy	no toxicologically relevant effect										
Organ weights (% of control-100) pituitary (abs.) liver (abs.) thyroid (abs.)			 -10 +18	 -4 0	 +18	 -6	 +5 +6	 +3 +13	 -29*** -12 +12	 -26*** +26*** +38	
Pathology	no toxicologically relevant effect										

dr = dose related; i = increased; d = decreased; ds = decreased significantly;
d (t) = temporarily decreased (first 4 weeks of study)
all incidental to blood-sampling stresses; * p<0.05; ** P<0.01 *** p<0.001 (students t-test, compared with control group, calculated by RMS)
[a] fraction (f) of value in blood after 13 weeks of treatment, as compared to value before treatment

Table 17. Body, liver and thyroid weight in a 90-day oral toxicity study in rats (DAR addendum 2010: Wheldon, 1974a)

	Males receiving (ppm):					Females receiving (ppm):				
	0	100	500	1000	20000	0	100	500	1000	20000
Body weight (g)	584	577	605	576	330	312	324	333	324	245
Liver weight (g)	25.8	24.0	26.1	25.9	22.6	13.7	12.6	13.6	13.6	17.3
change to control (%)		*-7*	*1*	*0*	*-12*		*-8*	*-1*	*-1*	*26*
Body weight-related liver weight (%)	4.4	4.1	4.3	4.5	7.1	4.3	4.0	4.1	4.2	7.2
change to control (%)		*-7*	*-2*	*2*	*61*		*-7*	*-5*	*-2*	*67*
Thyroid weight (g) $x10^3$	17	20	20	18	19	16	16	15	18	22
change to control (%)		*18*	*18*	*6*	*12*		*0*	*-6*	*13*	*38*
Body weight-related thyroid weight $x10^3$ (%)	3.0	3.5	3.3	3.2	6.0	5.2	5.1	5.0	5.6	9.2
change to control (%)		*17*	*10*	*7*	*100*		*-2*	*-4*	*8*	*77*

On the basis of effects on lower body weight gain in males and females, and increased thyroid and liver weights at 20000 ppm in females, the NOAEL was 1000 ppm (equal to 50-150 mg/kg bw/day).

The study deviated from the current guideline (OECD 408) in that the interval of the last 2 doses was 20-fold, instead of recommended 2 to 4 fold; no measurement for albumin, total protein,

creatine, urea nitrogen, cholesterol, Na and K was performed in blood and only 2 instead of 3 liver enzymes were analysed; no organ weight of uterus and epididymides were taken; no histopathology was performed on oesophagus, peripheral nerve, mammary glands, tracheas, aorta, skin and eyes. Despite these deviations, the study was considered to be acceptable.

In another subchronic toxicity study (DAR: Wheldon, 1974b), bupirimate (99.5% pure) was administered orally in gelatine capsules at dose levels of 0, 3, 15, 30 and 600 mg/kg bw/day daily for 13 weeks to 5 groups of 4 male and 4 female Beagle dogs. Dose administration was conducted after the 1st meal. Clinical observations were recorded daily and body weights weekly. Food residues were weighed and weekly consumption calculated. Ophthalmological examinations were made before the start of treatment and in weeks 4 and 13. Blood samples were taken for haematological examination from all animals before the start of treatment and in weeks 6 and 13. Blood and urine samples for biochemical assessment were taken from all animals before the start of treatment and in weeks 6 and 13. At the end of the treatment period all animals were killed and subjected to necropsy. Organ weights were recorded and a selection of tissues from all animals was examined microscopically.

At 600 mg/kg bw/day, bupirimate depressed food intake and body weight gain from about the 4rd week of treatment until termination. These effects were particularly marked and progressive in one male, which eventually died in the last week of treatment. Autopsy revealed a small spleen, raised black areas on the mucosal surface of the gall bladder, and an area of haemorrhagic mucosa in the ileum; the latter was associated with a semi-solid thrombus filling the lumen. Histopathological examination only revealed immature tested and a regressive change in the thymus. One further dog (a female treated at 3 mg/kg bw/day) also died, but this was not treatment related. Animals treated at 600 mg/kg bw/day intermittently showed salivation associated with dosing from week 2 and a leaner body conformation from week 6, although not all were affected to the same extent. In general, they appeared subdued in the latter weeks of the study. A slower rate of growth was observed after the first 3-4 weeks and food consumption was lower particularly for males from week 4 onwards. Growth performance was normal in the lower dosage groups.

Two of the 3 surviving males treated at 600 mg/kg bw/day showed some evidence of normocytic anaemia in week 13 (Hb values 11.6 and 12.1, vs. a control mean of 14.0), but otherwise there were no treatment-related changes in haematological parameters. There were no treatment-related macroscopic changes at necropsy. Relative brain, liver and kidney weight were higher at 600 mg/kg bw/day, for brain this was a probably consequence of the observed body weight retardation. The same applied for the decrease in absolute weight of heart and spleen in both males and females, lungs in females and epididymides in males, all in the 600 mg/kg bw/day group. Absolute and relative weights of gonads were decreased in the 2 and for adrenals increased in the 3 highest dose groups, both only in males. Thymus weights were decreased in males and females of the 30 and 600 mg/kg bw/day groups. Thyroid weights were increased in all treated groups, although quite some variation was observed between the individual animals. Increased relative thyroid weights outside the range of historical control data were observed in males at 15, 30 and 600 mg/kg bw/day and in 1 female at 600 mg/kg bw/day, when compared to the statistically determined range. However, the historical control range can also be determined by using the individual control data from the same studies; this reveals that the thyroid weights in the study were outside the historical control range in all dose levels. Histopathological evaluation concluded that testicular maturation was unequivocally delayed in some dogs given 30 or 600 mg/kg bw/day; spermatogenesis proceeds to the stage of secondary spermatocyte formation only. One male dog at 30 mg/kg bw/day showed thymic regression and in one treated at 600 mg/kg bw/day the thymus was lacking.

Table 18. Results of a 90-day oral toxicity study in dogs (DAR addendum 2010: Wheldon, 1974b)

dose (mg/kg bw/d)	0		3		15		30		600		dr
sex	m	f	m	f	m	f	m	f	m	f	
Mortality #	0/4	0/4	0/4	1/4 #	0/4	0/4	0/4	0/4	1/4	0/4	
Clinical signs											
salivation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	4/4	4/4	
subdued	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	4/4	4/4	
body weight gain											
% of control -100			+21	+6	+13	+30	+6	-3	-69**	-66**	
Food consumption											
(as % of control-100)									-46***	-11*	
Urinalysis	no toxicologically relevant effect										
Haematology											
Hb									-13%*		
PVC									-12%**		
Clinical Chemistry	no toxicologically relevant effect										
Ophtalmoscopy	no toxicologically relevant effect										
Organ weights											
(% of control-100)											
liver (abs / rel.)								-14 / -7	-16/+43	-2/+28	
kidney (abs / rel.)								-2 / +7	-21/+34	-17/+11	
thymus (abs / rel.)							-19/-24	-23/-18	-40/-16	-60/-46	dr
testes (abs / rel.)							-19/-15		-61/-45		dr m
adrenals (abs / rel.)					+24/+23		+27/+28	+13/+21	+49/+175	-5/+27	dr
thyroids (abs / rel.)			+30/+22	+25/+28	+42/+38	+39/+32	+49/+51*	+50/+58*	+8/+91*	+30/+60	dr
Histopathology											
Immaturity of testes											
- Immature	1/ 4		1/4		0/4		2/4		3/4		
- Possibly immature	0/4		0/4		3/4		1/4		0/4		
Thymus regression	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	1/4	0/4	

* p<0.05; ** P<0.01; *** p<0.001 (students t-test, compared with control group, calculated by RMS)
# not related to treatment

Various degrees of testis maturity were evident when the sections of testis were examined. The distribution of male dogs displaying any indication of sexual immaturity was 1, 1, 3, 3 and 3 in the respective dose groups. It should be realised that the dogs in the study were 33-38 weeks of age at the end of the study; hence any findings on maturity of testis should be assessed with caution. The findings at 15 and 30 mg/kg bw/day could still be considered comparable to controls; at 600 mg/kg bw/day ¾ animals showed unequivocal immaturity of testis, compared to ¼ in controls. However, even this apparent treatment-related effects should probably be considered secondary to the reduced growth of the treated animals. No effects on maturity of testis and/or other effects on reproduction were seen in male animals in the 2-year dog study, nor in the reproduction study in rats. The effects on testis in this dog study is thus considered not a relevant adverse effect.

The absolute and relative thyroid weights showed a rather clear dose response in males and females from 3 mg/kg b/day onwards. Therefore these data were compared to historical control data. Because of small groups, comparing data of individual animals has preference over comparing of group means. First of all it is remarkable that in the study the control values for males were very low; individual values (absolute and/or relative) are lower than the historical control range in ¾ males, as are the mean control values. For control females the absolute thyroid weight of 2 out of 3 females and the relative thyroid weight of 1 female are lower than the historical control range.

At 3 mg/kg bw/day males, the absolute thyroid weight of 1 male is slightly higher than the historical control range but the relative thyroid weight of this male is within the range. However, the absolute and relative thyroid weight of another male is lower than the historical control range. Group means of the males in this dose group are within the historical control range. At 3 mg/kg bw/day females, all data were within the historical control range. All male and female data are within the statistically determined historical control range.

At 15 mg/kg bw/day males, the absolute and relative thyroid weight of one male is higher than the historical control range. However, the absolute and relative thyroid weight of another male is lower than the historical control range. Group means of the males in this dose group are within the historical control range. At 15 mg/kg bw/day females, relative thyroid weight of one female is higher than the historical control range. Group means of the females in this dose group are within the historical control range. Similar results ware observed when comparing the male data to the statistically derived historical control range; however, all female data were within these historical control data.

Based on the effects observed in thymus (decreased organ weight and histopahology) the NOAEL was set to 15 mg/kg bw/day.

The study deviated from the current guideline (OECD 409) is that the interval of last 2 doses is 20-fold rather than recommended 2- to 4-fold; in blood, no measurement for albumin, total protein, creatine, urea nitrogen, Ca, P, Na and K was performed; no organ weight of uterus and gall bladder were taken, and no histopathology was performed on peripheral nerve, mammary glands, aorta, skin and eyes. Nevertheless, the study is considered to be acceptable.Chronic (2 year studies) are described in more detail in chapter 4.8. Some relevant information is provided here.

The average daily bupirimate intakes (mg/kg bw/d) of the oral 104 weeks-study in rats of Ben-Dyke et al (DAR: 1977a) were not presented in the report, but are calculated to be:

	100 ppm	1000 ppm	5000 ppm
Males			
Week 1	13.5	137.2	725
Week 26	3.6	35.3	189
Week 78	3.0	24.6	156
Females			
Week 1	13.2	130.3	769
Week 26	5.3	54.3	337
Week 78	3.4	32.3	252

Survival was only 8-28% in males and 23-40% in females at the end of the study; survival in each group was $\geq$ 50% until the 74th week of the study (Table 23). No treatment related effects were found on mortality. As the high mortality at the end of the study influenced the mean food consumption and body weight per group, bodyweight, food consumption and food conversion factors in Table 23 are shown only up to the 78th week. The body weight gain of rats of either sex receiving 1000 and 5000 ppm were reduced in a dose-related manner. Statistics were only performed for the first year of study. The food conversion efficiency was considerably lower in highest dosed animals of both sexes, but especially in the highest dosed females (no statistics performed).

Reductions in Hb, PVC and RBC in females receiving 5000 ppm were seen from week 26 onwards. One-off analysis of serum thyroxine levels in week 69 from 5 males and 5 females treated at 5000 ppm showed that in female the thyroxine level was 1.5 times that of control, but as the

statistical power is low with only 5 samples per sex and values varied from 19 to 81 nM/l, this was not a statistically significant difference.

In high dosed females the relative weights of brain, pituitary, heart, liver, kidney and ovaries were higher, while in high dosed males the relative weights of brain, liver and thyroid were higher than in controls. The increase in absolute and body weight-related mean thyroid weight at 89 weeks was partly a consequence of 7 males with follicular thyroid adenomas. Thyroid weight was also slightly higher than controls in females at this level. Pituitary weights were widely differing, due to the fact that approximately half of all rats had tumours in this organ. Other intergroup differences in organ weights were reported to be related to the differences in body weight, except the increase in the weight of the kidney in females and of the liver in both sexes, which cannot be explained only by the body weight differences, although no histopathological changes were found.

The decrease of body weight at 1000 ppm was considered not sufficiently biologically relevant due to absence of other effects. Based on the severly reduced (>20%) body weight at 5000 ppm, the NOAEL for this study was therefore set to 1000 ppm (25 mg/kg bw/d).

In the oral 105-week study in dogs (Ben-Dyke, 1977c), bupirimate at dosage of 200 mg/kg bw/d was associated with emesis and salivation. With the exception of the period between weeks 9 and 23, males receiving 200 mg/kg bw/d had reduced food intake (maximum of 14% less than controls over weeks 27-52). Females in the highest dosing group showed a decrease in food intake during the first 8 weeks of treatment but the overall food consumption in the 2 highest dosing groups was slightly higher than controls. Administration of bupirimate to male dogs was associated with a reduced weight gain, which was statistically significant at 200 mg/kg bw/d until the 78th week. In the low dose the decrease was only marginal and the range of body weights of the low dose animals was similar to that of the controls.

PCV, Hb and RBC concentration values were statistical significantly lower (mostly between -10 and -17% with respect to controls) in females receiving 20 and 200 mg/kg bw/d on several time points during treatment, and in females receiving 5 mg/kg bw/d only in week 13. Increases (up to 2 to 4 times) in serum alkaline phosphatase (20 and 200 mg/kg bw/d) and glutamate pyruvate transaminase activities (200 mg/kg bw/d) occurred in both male and female dogs. These changes showed some correlation with increases in liver weight and the presence of lipofuscin in some hepatocytes.

At necropsy, 3/8 animals receiving 200 mg/kg bw/d bupirimate had dark colouration of the liver. Body weight-related liver was increased in males and females receiving 20 and 200 mg/kg bw/d, and in males only of the 5 mg/kg bw/d group. The body weight-related thyroid weight was increased in both sexes at 200 mg/kg bw/d, in females at 20 mg/kg bw, and in males of the 5 mg/kg bw/d group.

Histopathological examination revealed treatment-related changes in the liver. Increased numbers of pigment-laden macrophages in the portal tracts or pigment containing Kupffer cells in the hepatic sinusoids were noted in 6/8 animals receiving 200 mg/kg bw/d and 1/8 animals receiving 20 mg/kg bw/d. The appearance of the pigment was typical of lipofuscin and, in two dogs receiving 200 mg/kg bw/d, the generalised degree of hepatic lipofuscin pigmentation was greater than normal. In a single female receiving 200 mg/kg bw/d there was a significant degree of hepatocytic cytoplasmic vaculolation, and in a single male receiving 200 mg/kg bw/d fibrosis of the liver was observed.

In summary. oral (capsule) administration to the beagle dog, daily for 105 weeks resulted in effects

on body weight, liver weight, serum liver enzymes and liver pathology at 200 mg/kg/day and lesser changes on serum liver enzymes and liver pathology at 20 mg/kg/day. The effects observed at 5 mg/kg bw/d were in the range of control values (body weight), not consistent over time (haematology), only marginal without concurrent findings in clinical chemistry/histopathology (liver weights), or not dose related (thyroid weights). Hence the NOAEL was considered to be 5 mg/kg/day.

4.6.1.2 Repeated dose toxicity: inhalation

This information is not available.

4.6.1.3 Repeated dose toxicity: dermal

In a subacute dermal toxicity study (DAR: Gore, 1975), 10 daily doses of bupirimate of unspecified purity were applied as an aqueous suspension (25% w/v "Dispersol"OG) to the shaven backs of 6 female New Zealand White rabbits 5 days/week for 2 weeks, at a single dose level of 500 mg/kg bw/day. An occlusive dressing was applied for 18 hours/day and, on each removal, the treated sklin was washed with methylated spirit. The animals were weighed daily. Blood was taken for haematological and biochemical assessment after the 5^{th} and the 10^{th} application. After the last application the animals were killed and the number of tissues were taken for histopathological examination. A control group was not included.

There were no deaths during the study. Dermal application of 500 mg/kg bw/day was associated with marked erythema in all animals. The erythema was speculated to be the result of vigorous decomtamination necessary to remove the test material from the skin; but there were no control data to support this. Animals failed to gain weight normally, and slight weight loss was seen in 4 of 6 animals. Blood urea levels were raised in 4 of 6 animals between the 5^{th} and 10^{th} application. No other biochemical or haematological changes were seen between 5^{th} and 10^{th} day. Histopathological examination revealed a moderate degree of kidney damage in 5/6 animals and a mild inflammator5y reaction in the liver of 3/6 animals.

Beause only one dose level was tested and a control group was lacking, the study is considered as supplementary.

4.6.1.4 Repeated dose toxicity: other routes

This information is not available.

4.6.1.5 Human information

No human information is available.

4.6.1.6 Other relevant information

This information is not available.

4.6.1.7 Summary and discussion of repeated dose toxicity

Sub-acute and semi-chronic oral studies in rat and dog were available. In these studies the main targets for bupirimate, seen in both rat and dog, were bodyweight, liver, kidney and thyroid. Reduction in body weight gain was accompanied, although to a lesser extent, by a reduction in food consumption. Increases in liver, kidney and thyroid weights occurred in the absence of histopatological changes. In dogs only, immaturity of testes, increased adrenal weight and thymus regressions were also seen. The 90-day study in the dog provided the lowest short-term LOAEL (30 mg/kg bw/day), based on decreased thymus weight, accompanied by histopathology.

Chronic studies are available in rat, mice and dogs and are summarised in chapter 4.8. Non-neoplastic effects observed in the chronic rat study included reduced bodyweight gain and reduced food conversion in both sexes at 25 and 156 mg/kg bw/day. Reductions of Hb, PVC and RBC were reduced in female rats at 125 mg/kg bw/day. Also increased relative weights for some organs were observed at the highest dose level. Increased thyroid weight may be related to tumour formation (also see 4.8). The study in mice was considered not acceptable due to various limitations but did not indicate non-neoplastic effects at the studied dose levels.

The much shorter 10-day gavage studies in rats show effects at 250 and 1000 mg/kg bw/day including incontinence especially in females. This effect was also observed in the developmental toxicity study with rats (gavage) at dose levels of 50 (incidence 2/22), 150 (10/23) and 400 mg/kg bw/day (21/22). Urinary incontinence was seen in high-dosed rats but only by gavage (bupirimate), not via diet.

Oral (capsule) administration to the beagle dog, daily for 105 weeks resulted in effects on liver weight, serum liver enzymes and liver pathology at 20 mg/kg bw/day. The effects observed at 5 mg/kg bw/day were not consistent over time (haematology), only marginal without concurrent findings in clinical chemistry/histopathology (liver weights) or not dose-related (thyroid weights).

The available 10-day dermal study is considered as supplementary due to the absence of a control group and indicates some moderate kidney damage and a mild inflammatory reaction to the liver at 500 mg/kg bw/day (only dose tested).

4.6.1.8 Comparison with criteria

According to the EC classification criteria, the substance has to be classified for repeated dose toxicity, if significant toxic effects, relevant for human health, are observed at dose levels ≤ 100 mg/kg bw/day (CLP) or ≤ 50 mg/kg bw/day (DSD) in oral 90-day studies with rats. Based on the results of the available 90-day study, in which lower body weight gain in males and females, and increased thyroid and liver weights were the only effects observed only at the highest dose level of 20000 ppm (estimated daily intakes in the range of 1698 – 2523 mg/kg bw/day for males and 2270 – 2574 mg/kg bw/day for females), these criteria are not fulfilled.

The much shorter 10-day gavage studies in rats showed effects at 250 and 1000 mg/kg bw/day including incontinence especially in females. Incontinence could indicate a functional impairment. However, it could also be that this is just another description for the peri-genital staining that is reported more commonly in rats as a clinical effect. This could be an indication of stress. With regard to the DSD criteria it is important to note that the effect was reversible. Further, this effect was only observed in gavage studies and not in diet studies. Applying Haber's rule to extrapolate equivalent guidance values for a 10 day study (CLP Annex I 3.9.2.9.5) result in a guidance value of 900 mg/kg bw/day (CLP) and 450 mg/kg bw/day (DSD). The presence of incontinence in the developmental study (exposure day 7 to 16) also occurred at dose levels (50 mg/kg bw/day and

above) that are relevant even for exposure durations up to 90 days (100 mg/kg bw/day). Although incontinence was observed at dose levels relevant for classification for STOT-SE, the effect is not considered as significant and specific target organ toxicity and probably more related to stress.

The oral 28-day study in male rats focussing on the effects on the thyroid shows that at levels of 500 mg/kg bw/day (lowest dose tested) and above there is a clear effect on the thyroid. However, there is no indication that such effects occur at lower dose levels relevant for classification. Further, rats are considered to be more sensitive to disturbance of the thyroid hormone axis (see carcinogenicity) than humans.

No non-neoplastic effects were observed in the chronic rat study at dose levels relevant for classification concerning a 2-year study (6.25 (DSD) and 12.5 (CLP) mg/kg bw/day).

No guidance values are provided for the 90-day and 2-year oral studies in dogs. The effects at 30 mg/kg bw/day in the 90-day study were limited decreased thymus weight in males and females and thymus regression in 1 out of 4 males but not in females. At the much higher next dose level of 600 mg/kg bw/day, much stronger effects were observed clearly fulfilling the criteria. Estimating the effects at the guidance level that are applicable to rats (50 and 100 mg/kg bw/day) and assuming they are also acceptable for dogs requires interpolation between 30 and 600 mg/kg bw/day. As these guidance values are much closer to 30 mg/kg bw/day, it is expected that the effects at these guidance values are limited and do not warrant classification.

The limited liver effects in the 2-year study dogs at 20 mg/kg bw/day do not warrant classification.

The effects observed in the available 10-day dermal study would fulfil the classification criteria. However, due to the limitations of the study a conclusion cannot be drawn based on only this study.

4.6.1.9 Conclusions on classification and labelling

The available oral studies do not show significant toxic effects at dose levels requiring classification as STOT-RE.

The results of the limited dermal study contradict the results of the oral studies especially when taking the limited dermal availability into account (chapter 4.1). Therefore, no classification is required for effects after repeated dermal exposure.

No classification for effects after repeated inhalatory exposure is required due to absence of data.

Table 19 Conclusion on classification for repeated dose toxicity

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Not classified	Not classified

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.

Additional key elements

The DS added the following clarifications in relation to the thymus-related effects in the dog studies:

2-year dog study:

Microscopic examination of the dogs included the thymus. There was no statistical significant effect on absolute or relative thymus weight. It should be noted that the thymic weight showed a high interindividual variation. No macroscopic or histopathological changes of the thymus were observed.

90-day dog study:

The relative thymus weight in the 30 mg/kg bw/day group (males) was reported correctly including for animal 416 with thymic regression (thymus weight: 0.4 g). The relative thymus weight in the 600 mg/kg bw/day dose group (males) was however incorrectly reported in the original study report. The correct average relative thymus weight was -37% of the controls when including the dog without thymus (animal 430, see the table below). In addition, it was stated in the study report that animal 416 of group 4 (male) had a low thymus weight with histological evidence of thymic regression. However, the histopatological changes were not described.

The original study report does not contain information regarding statistical significance. The rapporteur conducted a student's t-test on thymus weights which showed no statistically significant changes in males (but there was high variability). For females, the thymus weights were significantly reduced at 30 mg/kg bw/day (absolute) and at 600 mg/kg bw/day (absolute and relative).

It was stated in the report that dogs showed a normal age related thymus involution as stated, a statement supported by the lower thymic weights in the 2-year study.

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 Studies	STOT RE 1	STOT RE 2	Dose-response data
Rat 10 d	90	900	Dose levels tested: 250-1000 mg/kg/d 250 mg/kg/d and 1000 mg/kg/d: Incontinence especially in females. The DS does not consider this effect as significant or specific target organ toxicity. The effect was considered probably more related to stress.

Rat 90 d	10	100	Dose levels tested: 4.2-24-49-1698 mg/kg/d 49 mg/kg/d: NOAEL 1698 mg/kg/d: Effects not warranting classification: lower body weight gain in males and females and increased thyroid and liver weights in the absence of histopathological changes.
Rat 2 y	1.25	12.5	Dose levels tested: 3-24.6-156 mg/kg/d 24.6 mg/kg/d: NOAEL 156 mg/kg/d: Effects not warranting classification: severely reduced body weight gain and increased relative weights of different organs in the absence of histopathological changes.

Dog studies	STOT RE 1	STOT RE 2	Dose-response data
Dog 90 d	10	100	Dose levels tested: 3-15-30-600 mg/kg/d Testicular maturation: the dogs selected in this study were 33-38 weeks of age at the end of the study; because they were still juveniles, any findings on maturity of testis should be assessed with caution. Furthermore, at doses with reduced growth of treated animals immaturity of testis can be considered secondary to reduced body weight gain. No effects on maturity of testis were seen in male animals in the 2-year study in dogs, and no effects on reproduction were seen in the reproduction study in rats. Therefore the effect on the testis in the 90-day oral dog study is not considered a relevant adverse effect. 15 mg/kg/d as NOAEL. Increase in adrenal and thyroid gland weight without histological findings. 30 mg/kg/d: Increase in adrenal and thyroid gland weight without histological findings; Statistically significant reduction of absolute (not relative) thymus weight only in females; no reporting of specific histological changes in the regressed thymus. 600 mg/kg/d as effective dose: various adverse effects including testis pathology. More pronounced decrease of thymus weight. Thymus was lacking in 1/4 males. No histopathological changes in the thymus are reported.

Dog 2 y	1.25	12.5	Dose levels tested: 5-20-200 mg/kg/d 5 mg/kg/d: NOAEL 20 mg/kg/d: changes on serum liver enzymes, liver pigmentation in 1/8 animals. Haemogliobin reduction less than 20%. 200 mg/kg/d: Effective dose: effects on body weight, liver weight, serum liver enzymes and liver pathology (lipofuscin pigmentation). Haemogliobin reduction less than 20%. Additional information on thymus toxicity Thymic weight showed large variation. No statistically significant effect on absolute or relative thymus weight was observed. Macroscopic or histopathological changes of the thymus were not observed.

Thymus findings in the 90-day oral toxicity study in dogs are presented in the table below.

Table: Thymus findings in the 90-day dog study (CLH-report, table 18)

Dose	0	0	3	3	15	15	30	30	600	600
Sex	M	F	M	F	M	F	M	F	M	F
BWG* in % of control			+21	+6	+13	+30	+6	-3	-69	-66
Thymus weight (abs/rel)							-19/-24** n.s.	-23/-18 abs: s. rel: n.s.	-40/-16*** corrected: -40/-37% n.s.	-60/-46 abs: s. rel: s.
Thymus regression	0/4	0/4	0/4	0/4	0/4	0/4	**1**/4 thymus regression without histological charac-terisation	0/4	**1**/4 thymus lacking	0/4

* body weight gain; ** the thymus weight is reported correctly, the calculation includes the animal with thymic regression; *** the relative thymus weight in males accounted only for 3 of the 4 males; integrating the animal without thymus results in a corrected average relative thymus weight of -37% of the controls. s. = significant; n.s. = not significant.

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.

4.7 Germ cell mutagenicity (Mutagenicity)

Table 20: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Test details	Results	Study quality (Klimisch score)	Reference
In vitro Ames test, *Salmonella typhimurium* Resembled OECD 471, No GLP	Strains TA98, TA100, TA1535, TA1537 and TA1538 0 – 2500 µg/plate	Negative (+/- S9)	Not reliable: misses strains	DAR (Trueman,, 1979)
DNA repair test No guideline No GLP	*E.coli* W 3110/pol A^+ and A^- Direct plating: 0-3.3 mg/plate Suspension: 0 and 10 mg/plate	Negative (+/- S9)	Not reliable	Riach *et al.* (1979)
In vitro Ames test, *Salmonella typhimurium* OECD 71 GLP	3.16-316 µg/plate	Negative (+/- S9)	Reliable	DAR addendum 2010 (Flüge, 2009)
Mammalian cell transformation test No guideline No GLP	Baby Hamster Kidney Fibroblasts (BHK 21/C13) 0, 0.25, 2.5, 25, 250, 2500 µg/mL	Negative (-S9) Dose-related increase (+S9)	Not reliable	DAR (Trueman,, 1979)
In vitro chromosome aberration test, human lymphocytes (two donors) OECD 473 GLP	Donor 1: 5.8-58.6 µg/mL Donor 2: 5.8-31.3 µg/mL; 1.5-31.3 µg/mL (repeat test)	Donor 1: negative (-S9), positive* (+ S9) Donor 2: negative –(S9), positive* (+ S9), negative (+ S9, repeat test)	Reliable without restrictions	DAR (Howard, 1989)
In vitro DNA repair assay, human embryonic intestinal fibroblasts Resembled OECD 482 No GLP	7.81-1000 µg/mL	Negative (+/-S9)	Reliable with restrictions	DAR (Poole & Harris, 1979)
In vitro gene mutation in mammalian cells (L5178Y mouse lymphoma cells) OECD 476 GLP	0-200 µg/mL	Negative (+/-S9)	Reliable without restrictions	DAR (Callander & Clay, 1992)
In vivo mouse micronucleus test OECD 474 GLP	Males: 0, 2370 and 3800 mg/kg bw; females: 0, 2070 and 3320 mg/kg bw; assessed at 24, 48 and 72 hours	Negative	Reliable without restrictions	DAR (Randall & Mackay, 1989)
In vivo dominant lethal	Males only: 0, 30, 300	Negative**	Reliable with	DAR

assay in mice No guideline\No GLP	and 3000 mg/kg bw/day		restrictions	(McGregor, 1976)

* Only positive at dose levels associated with cytotoxicity

** Precipitation of bupirimate

4.7.1 Non-human information

4.7.1.1 In vitro data

Two gene mutation studies with Salmonella typhimurium were available. One of the studies, performed by Trueman (DAR: 1979), was judged to be not suitable for evaluation, as the tests were not conducted at a high dose level associated with cytotoxicity or solubility problems with bupirimate, and the highest dose level was below the recommended limit of 5 mg/plate. Furthermore, the substance was not tested on the recommended strain *S. typhimurium* TA102, or *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), and the tests were carried only in the presence of S9. However, negative results were observed in all cases in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 up to concentration levels of 2500 µg/plate. The study is considered supplementary.

In the second study of Flüge, (DAR: 2009), performed according to OECD Guideline 471, bupirimate was assayed in triplicate in *S. typhimurium* strains TA98, TA10, TA102, TA1535 and TA1537, using the direct plate incorporation method (1^{st} independent experiment) or the preincubation method (2^{nd} independent experiment), both with and without metabolic activation. DMSO was used as a vehicle. Precipitation was observed at dose levels ≥ 316 µg/plate. Toxicity was not observed. The test substance did not induce point mutations in *S. typhimurium.* The study was considered acceptable.

Bupirimate was tested for the potential to affect DNA repair with two *E. coli* strains W 3110/pol A+ and p3478/pol A-, first in a direct plating method (0-3.3 mg/plate) followed by a suspension test to overcome the problem of low solubility of bupirimate (0 ad 10.0 mg/plate) (DAR: Riach, 1979). Studies were not performed according to any guideline as only 2 bacterial strains were used, only one concentration was tested in the most reliable suspensions test, no individual data and statistical evaluation were reported. The study is considered not acceptable.. In these tests bupirimate did not produce DNA-damage, with and without metabolic activation.

Bupirimate was tested in a mammalian cell gene mutation test using mouse lymphoma cells L5178Y TK+/- according to OECD Guideline 476 and EPA guidelines (DAR: Callander & Clay, 1992). Concentrations of bupirimate were selected from a dose-range finding study. The highest doses were selected on the basis that they were freely soluble and produced significant toxicity. Three independent experiments were carried out. In these experiments dose-related toxicity was seen within a steep dose-response relation. Bupirimate induced no significant dose-related or reproducible increases in mutant frequency when examined over a range of concentrations that reduced cell survival by up to 93% in the presence and absence of S9. Under the test conditions, bupirimate was not mutagenic to L5178Y TK+/- cells, with and without metabolic activation, when tested up to dose levels (180 µg/mL) limited by toxicity. The study is considered acceptable.

The mammalian cell transformation assay was performed with bupirimate using Baby Hamster Kidney Fibroblasts (BHK 21/C13) (DAR: Trueman, 1979) using concentration ranges 0-2500 µg/mL. Bupirimate caused increasing cell mortality with increasing dose. The LC50 was determined to be 99 µg/mL. At this LC50, the corrected transformation frequency was calculated to be $223/10^6$ survivors. This value was more than 5 x the spontaneous count of $19/10^6$ survivors

indicating that bupirimate was positive in the test system.The study was not performed according to any guideline. The value of the study was limited, as it was tested below maximal levels. Furthermore study results were poorly described. Therefore the study was considered to be not acceptable for evaluation.

Poole and Harris (DAR: 1979) reported a DNA repair assay in non-dividing cultured human intestinal fibroblasts. The protocol resembles OECD guideline 482. In a preliminary toxicity study (0-1000 µg/mL), bupirimate showed to be water soluble and relatively non-toxic to cells at levels of and below 100 µg/mL. At 1000 µg/mL, bupirimate precipitated from solution forming an uneven deposit and cells in the vicinity were killed. The dose of 1000 µg/mL was used as the highest concentration in the main DNA repair studies.

In the first main study incorporation of ^{3}H-thymidine was measured in 2 independent tests. In the 1st test, lower +3H-thymidine uptake was found in the bupirimate cultures with S9 than in the solvent controls, while in the 2nd test there was no evidence of DNA repair synthesis in bupirimate cultures with or without S9. Because of these negative results, a different test system was conducted to assess the incorporation of ^{3}H-deoxyguanosine to look at the repair of guanine residues. At 1000 µg/L bupirimate, no evidence of DNA repair was seen with or without S9. Unexpectedly, there was some radioactivity incorporation in the fractions from the solvent controls without S9, although at levels below that of the positive controls. The validity of the test must be considered doubtful with such unusual control results.

In 1979 no guidelines were available for assessment of mutagenic potential. However, the study was based on a modification of the method which resembled OECD guideline 482. The ^{3}H-thymidine method showed only minor deviations from OECD 482 (3 instead of 6 cell cultures/concentration, cpm/coverslip instead of dmp/ µg DNA) and can therefore be used for evaluation of genotoxicity. In the ^{3}H-deoxyguanosine method 1 instead of 6 cell cultures per concentration was tested. Additionally, some unusual control results were obtained. The study results are therefore considered not acceptable for evaluation.

A cytogenetic assay in human lymphocytes was performed according to OECD guideline 473 by Howard (DAR: 1989). Lymphocytes from 2 healthy volunteers (donor 1, male, and donor 2, female) were used. The appropriate dose levels for chromosomal analysis in the main study were selected from a cytotoxicity test using a range of bupirimate dilutions up to 312.5 mg/mL (limit of solubility). Furthermore, the highest dose levels selected for chromosomal analysis in the main study were based on an observed reduction of mitosis within the range 50-80%. The resulting dose ranges were 0, 5.8, 23.4, 58.6 µg /mL for donor 1, 0, 5.8, 15.6, 31.3 µg/mL for donor 2 and 0. 1.5, 5.8, 15.6 and 31.3 µg/mL in the repeat experiment with donor 2. In the main study, small, but statistically significant increases in chromosomal damage were observed in some bupirimate cultures without metabolic activation. With donor 1 an increase was seen at the high dose level only, and with donor 2 at all three initial dose levels (not dose-related). The elevated levels of chromosomal damage were associated with severe cytotoxicity, manifest as perturbed chromosome morphology and indistinct chromosome staining. As a result of this cytotoxicity it was not possible to count 100 cells for each culture (donor 2; one replicate from the 2 highest dose levels < 15 countable cells). The experiment was therefore repeated for donor 2 adding an additional dose level of 1.5 µg/mL (-S9). In this repeat experiment, cytotoxicity in terms of depression in mitotic index was as in the 1st experiment, but the other changes (perturbed chromosome morphology and indistinct chromosome staining) were not apparent. In this 2nd experiment, no increase in chromosomal aberrations over controls was seen.

When the 1st experiment was conducted in the presence of S9, there was no indication of an increase in chromosomal damage in the bupirimate groups.

Under the test conditions, bupirimate did induce chemically mediated clastogenicity in cultured human lymphocytes when tested at dose levels up to 58.6 µg/mL with metabolic activation. Results were not reproducible in a repeat test with donor 2. The study is considered acceptable.

4.7.1.2 In vivo data

Randall and Mackay (DAR: 1989) performed a mouse micronucleus test according to OECD 474 and EPA guidelines. From a dose-range finding study, the median lethal dose (MLD) was estimated by logistic regressions as 4749 mg/kg bw for male and 4144 mg/kg bw for female mice. 50% and 80% of the MLD were selected for the main study, resulting in the dose levels of 0, 2370 and 3800 mg/kg bw for males and 0, 2070 and 3320 mg/kg bw for females. The test substance was administered by a single oral exposure (gavage). 5 males and 5 females were used per dose group and per sacrifice time. In the main study, exposure to the low dose (50% MLD) was associated with one death in each sex. Exposure to the high dose (80% MDL) was associated with the deaths or premature sacrifice of 4 females. Clinical signs observed were subdued behaviour, hunched posture, urinary incontinence, eye discharge and tremors.

When the data were pooled, there were no statistically significant increases in the incidence of micronucleated polychromatic erythrocytes (MPE_ compared to vehicle controls at either dose level. When the sexes were considered separately, a small but statistical significant increase over the control value was noted in females 24 hours after being dosed at the high dose. This increase in MPE was considered to be without biological significance since its statistical significance was only by virtue of a low control value; examining a further 2000 polychromatic erythrocytes did not confirm the result; and the incidence of MPE was within the control data range for the males. The PCE/NCE ratio was statistically significant decreased in males in both dose groups (all sample times, except 24 hours at the low dose group), and in females in the highest dose group at 72 h after dosing indicating a toxic effect on the bone marrow.

Under the test condition, bupirimate was not clastogenic when assessed in the mouse bone marrow micronucleus test following oral (gavage) administration of a single dose of 2370 and 3800 mg/kg bw in male and 2070 and 3320 mg/kg bw in female mice. The study is considered acceptable.

A dominant lethal study in mice (15 males/dose) with bupirimate was conducted (DAR: McGregor, 1976). Mating was performed for 8 consecutive weeks; 2 untreated virgin females were paired with a treated male per week. The dose levels were selected on the basis of a 5 days range-finding study that utilised dose levels of up to 5000 mg/kg bw/day. The highest dose level at which deaths did not occur was 3000 mg/kg bw/day. In the main study, administration of bupirimate did not affect the frequency of pregnancy. The dose levels were 0, 30, 300 and 3000 mg/kg bw/day. In the group treated with 3000 mg/kg bw/day, the mean total number of implantations was significantly decreased in weeks 1 and 8, consequently also affecting the parameter – mean live implants and late deaths per pregnancy. In week 1, the low mean with bupirimate was a partial consequence of 2 of the pregnancies being single implantations; a finding that was seen in 1 control pregnancy in the same week, and was consequently considered unlikely to be an effect of treatment. The low week 8 mean actually fell within the range of control means and was also considered unlikely to be a treatment effect. At 30 and 300 mg/kg bw/day, there were higher incidences of transformed early deaths per pregnancy in week 1. There was no effect on early deaths with bupirimate at 3000 mg/kg bw/day so this finding at the lower doses was not considered a treatment effect

In 1976, no OECD guidelines were available. That time, the dominant lethal test was one of the 3 test systems suggested by the report of the US Advisory Panel on Mutagenicity of Pesticides (1969) and was one of those recommended by a WHO Scientific Group (1971) concerned with the evaluation and testing of drugs for mutagenicity.

Oral administratin of bupirimate to the male mouse at dose levels up to 3000 mg/kg bw/day daily for 5 days did not affect the frequency of pregnancy during 8 consecutive weeks of mating. There was a slightly lower implantation rate at 3000 mg/kg bw/day in weeks 1 and 8 only but considered unrelated to treatment, and no indication of an effect on dominant lethal mutations.

4.7.2 Human information

No human information is available.

4.7.3 Other relevant information

This information is not available.

4.7.4 Summary and discussion of mutagenicity

Bupirimate was negative in an *in vitro* mutagenicity test on *S. typhimurium* (point mutations). Also a negative *in vitro* gene mutation assay on L5178Y mouse lymphoma cells is available. An *in vitro* DNA repair assay on human embryonic fibroblast cells was negative. An *in vitro* DNA repair assay on two E. coli strains and a hamster kidney fibroblasts transformation assay were not considered suitable for evaluation. Positive results were obtained in an *in vitro* chromosome aberration assay on human lymphocytes indicating a clastogenic potential for bupirimate. However, clastogenicity was not seen *in vivo* in the mouse micronucleus test, which overrules the positive chromosome aberration test *in vitro*. *In vivo* bupirimate did not induce dominant lethal mutations in mice. From these results it is concluded that bupirimate is not genotoxic.

4.7.5 Comparison with criteria

Based on the results of the available genotoxicity studies, classification of bupirimate is not warranted according to EC criteria.

4.7.6 Conclusions on classification and labelling

Table 21 Conclusion on classification for genotoxicity

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Not classified	Not classified

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.

4.8 Carcinogenicity

Table 22: Summary table of relevant carcinogenicity studies

Method	Test details	Results (NOAEL/LOAEL)	Study Quality (Klimisch score)	Reference
104 weeks oral diet, rat Resembled OECD 453 No GLP	40/sex /group, Dose: 0, 100, 1000 and 5000 ppm	NOAEL = 3 mg/kg bw/day (100 ppm) LOAEL = 25 mg/kg bw/day (1000 ppm) Thyroid follicular adenoma ↑ (male) skin fibroma increased in female rats	Reliable with restrictions	DAR (Ben-Dyke et al., 1976a, 1977a)
80 weeks oral diet, mouse No guideline No GLP	40 /sex / group Dose: 0, 100 and 1000 ppm	No evidence of carcinogenicity	Not reliable	DAR (Ben-Dyke et al., 1977b)
105 weeks oral gavage, dog Resembled OECD 452 No GLP	4 /sex /group Dose:0, 5, 20 and 200 mg/kg bw/day	NOAEL = 5 mg/kg bw/day LOAEL = 20 mg/kg bw/day No evidence of carcinogenicity	Reliable with restrictions	DAR (Ben-Dyke et al., 1977c)

4.8.1 Non-human information

4.8.1.1 Carcinogenicity: oral

Bupirimate (purity 95.5-99.0%) was given by dietary admixture at concentrations 0, 100, 1000 and 5000 ppm to CD rats (40/sex/dose) daily for 104 weeks (DAR: Ben-Dyke *et al*., 1976a, 1977a). Diets were prepared weekly. Clinical observations were recorded, body weight was recorded weekly for the first 4 weeks and every 2 weeks thereafter. Cage food consumption was recorded weekly and food conversion ratio was determined. Water consumption was assessed visually. Ophthalmic examinations were conducted on all animals before the start of treatment and then the eyes of 25 males and 25 females from each of the control and 5000 ppm groups were examined in weeks 26 and 52. The eyes of all surviving control and 5000 ppm animals were examined in weeks 78 and 104. Before the start of the treatment and after 14, 26, 51, 77 and 102 weeks, blood samples for haematological and biochemical analysis were taken from 10 males and 10 females of each group. In addition, in week 69, blood samples for measurement of serum thyroxine levels were taken from 5 males and 5 females from the control and 5000 ppm group. Overnight (16-hour) urine samples were collected from 10 males and 10 females from each group before the start of treatment and during weeks 13, 26, 49, 75 and 102. After 104 weeks all surviving animals were necropsied. Organ weights were recorded. Organ weights were also analyzed of all animals killed after 88

weeks, to ensure a reasonable sample size. A range of tissues from all animals was examined microscopically. To further investigate observed changes in the thyroid gland, up to a further 5 additional sections were assessed from all males.

Males receiving 100 ppm showed poor survival and survivors in this group were sacrificed in week 96. The average daily bupirimate intakes were not presented in the report, but were calculated to be as follows:

Males:

Week 1: 13.5, 137.2 and 725 mg/kg bw/day

Week 26: 3.6, 35.3 and 189 mg/kg bw/day

Week 78; 3.0, 24.6 and 156 mg/kg bw/day

Females:

Week 1: 13.2, 130.3 and 769 mg/kg bw/day

Week 26: 5.3, 54.3 and 337 mg/kg bw/day

Week 78: 3.4, 32.3 and 252 mg/kg bw/day

Table 23. Results from 104 weeks oral toxicity study in rats (DAR: Ben-Dyke *et al.*, 1976a, 1977a)

Dose (ppm)	0		100		1000		5000		dr
Sex	m	f	m	f	m	f	m	f	
Cumulative mortality									
at week 26	1/40	1/40	1/40	1/40	0/40	1/40	0/40	3/40	
at week 52	4/40	5/40	7/40	2/40	4/40	5/40	2/40	11/40	
at week 78	16/40	11/40	24/40	8/40	15/40	8/40	10/40	13/40	
at week 104	37/40	26/40	40+/40	24/40	30/40	25/40	29/40	31/40	
Clinical signs	no toxicologically relevant effect								
body weight									
at 52 wk (% of control-100)			+4	-5*	-5	-8**	-16***	-28***	dr
at 78 wk (% of control-100)				-8#	-6#	-6#	-16#	-34#	dr
Body weight gain									
(0-78 wks, % of control-100)				-9#	-7#	-8#	-18#	-40#	dr
Food consumption									
(0-78 wks, % of control-100)			+5		-4		-9	+6	
Food conversion ratio									
(0-78 wk, % of control-100)			-	+9	-	+10	+11	+77	dr
Ophthalmoscopy	no toxicologically relevant effect								
Haematology									
PVC								ds	
Hb								ds	
RBC								ds	
Blood chemistry									
thyroxine			n.d.	n.d.	n.d.	n.d.		i	
Urinalysis	no toxicologically relevant effect								
Organ weights$ (% of control-100)									
relative kidney			-15	+13*	-21	+7	-10	+33**	
relative liver			-9	+8	0	0	+14	+25***	dr
relative thyroid			0	+6	+7	-14	+50*	+24	dr m
relative pituitary			+205	+16	+124	+41	+79	+103*	dr f
Total number of tumours	no toxicologically relevant effect								
Total number of tumour-bearing rats	no toxicologically relevant effect								

Dose (ppm)	0		100		1000		5000		dr
Sex	m	f	m	f	m	f	m	f	
Pathology									
Mammary tissue:									
Adenocarcinoma		1		2		2		5	
Skin and subcutis:									
Fibroma	0	1	0	1	3	1	5	5*	
Thyroid:									
Follicular adenoma	1	0	2	1	2	0	11*	2	

ds = decreased significantly, i = increased, dr = dose related, n.d.= not determined
+ only 3 animals surviving at week 96, so group terminated
* p<0.05; ** p<0.01 *** p<0.001 (compared with control group, Analysis of variance, except for Pathology, where the Fisher's exact probability test is used);
# no statistical tests performed
food conversion ratio = g food / g bodyweight gain
$ organ weights from all rats surviving at least 88 weeks of treatment are included (n=11-23 in male, n=25-29 in female).

The results of the study are shown in table 23. General toxicity effects are described in 4.6.1.1 (on repeated dose toxicity).

In high dosed females the relative weights of brain, pituitary, heart, liver, kidney and ovaries were higher, while in high dosed males the relative weights of brain, liver and thyroid were higher than in controls. The increase in absolute and body weight-related mean thyroid weight at 89 weeks was partly a consequence of 7 males with follicular thyroid adenomas. Thyroid weight was also slightly higher than controls in females, at this level. Pituitary weights were widely differing, due to the fact that ca. half of all rats had tumours in this organ. Other intergroup differences in organ weights were reported to be related to the differences in body weight, except the increase in the weight of the kidney in females and of the liver in both sexes, which cannot be explained only by the body weight differences, although no histopathological changes were found.

In a high number of decedents (particularly males), some tissues were noted as autolysed at macroscopic or microscopic examination; which limited their availability for evaluation. Notably, fewer tumours were detected in males with autolysed tissues than those killed prematurely or sacrificed at term. A dose-related and statistically significant incidence of thyroid follicular adenoma was found in males at 5000 ppm. The incidence of subcutaneous fibromas was also significantly higher than controls in rats of 5000 ppm group ($p < 0.05$ for females and for sexes combined). Finally, in females of the highest dose group, the incidence of adenocarcinomas in the mammary gland seemed slightly elevated.

In week 69 serum thyroxine levels were measured in 5 males and 5 females from the control and 5000 ppm groups. The results indicate a wide range of values from rats of that age in controls, and no differences were detected between treated and control rats of either sex.

The study deviated from OECD 453 guideline requirements in that 40 instead of 50 rats were used per group; survival was considerably below the required 50% in each group at the end of the study. Nevertheless, the study is considered acceptable.

Necropsy and subsequent histopathology revealed a statistically significant increase in the incidence of subcutaneous fibromas (females). Since this incidence was just outside the historical control data range (Makhteshim Chemical Works Ltd., 2006), the relevance of this finding is doubtful. Increased incidences of follicular adenomas of the thyroid were found in males receiving 5000 ppm and 1000 ppm, reaching statistical significance only in 5000 ppm. The increased incidence occurred only with benign tumours, no malignant thyroid tumours were present. In addition, in female rats of the highest dose group, the incidence of adenocarcinomas in the mammary gland was slightly elevated, however, as this incidence was within the historical control data, it was considered a fortuitous finding.

Table 24: Comparison of study tumour incidence (Ben-Dyke et al, 1976a, 1977a) with HCD

Dose level ppm		***0***	***100***	***1000***	***5000***
Males	***No. animals***	***40***	***40***	***40***	***40***
Study data	*Mammary adenocarcinoma*	*0*	*1 (2.5%)*	*0*	*0*
HCD	*'73 to '74*	*0 - 2 (0.0 - 4.0%)*			
HCD	*'75 to '77*	*0 - 2 (0.0 - 3.1%)*			
HCD	*'77 to '79*	*0 - 1 (0.0 - 1.7%)*			
Study data	*Skin fibroma*	*0*	*0*	*3 (7.5%)*	*5 (12.5%)*
HCD	*'73 to '74*	*0 - 2 (0.0 - 5.7%)*			
HCD	*'75 to '77*	*0 - 17 (0.0 - 24.3%)*			
HCD	*'77 to '79*	*0 - 13 (0.0 - 20.3%)*			
Females	***No. animals***	***40***	***40***	***40***	***40***
Study data	*Mammary adenocarcinoma*	*1 (2.5%)*	*2 (5%)*	*2 (5%)*	*5 (12.5%)*
HCD	*'73 to '74*	*0 - 3 (0.0 - 6.3%)*			
HCD	*'75 to '77*	*0 - 8 (0.0 - 13.3%)*			
HCD	*'77 to '79*	*2 - 8 (3.0 - 13.3%)*			
Study data	*Skin fibroma*	*1 (2.5%)*	*1 (2.5%)*	*1 (2.5%)*	*5 (12.5%)*
HCD	*'73 to '74*	*0 - 2 (0.0 - 4.3%)*			
HCD	*'75 to '77*	*0 - 4 (0.0 - 6.8%)*			
HCD	*'75 to '77 M+F combined*	*0 - 19 (0.0 - 13.8%)*			
HCD	*'77 to '79*	*0 - 6 (0.0 - 9.0%)*			
HCD	*'77 to '79 M+F combined*	*2 - 19(0.0 - 14.4%)*			

Figures denote the number of tumours with the percentage incidence in brackets, e.g. 1 (2.5%)

Table 24 illustrates that the mammary adenocarcinomas reported in the Ben-Dyke et al, (1976a,

1977a) were within the range of the contemporary HCD of the same laboratory (Huntingdon Life Sciences – HLS, HCD; 1973-1979). Additionally the table shows that skin fibromas for males and the sexes combined were also within the range of the HCD. The incidence of skin fibroma for females was close but just outside the HCD.

Decreases in body weight gain were dose-related and seen in both sexes at 1000 and 5000 ppm, although the effect at 1000 ppm is only slight. Based on the increased thyroid tumour incidence in males at 1000 ppm, the NOAEL was concluded to be 100 ppm (equal to 3 mg/kg bw/day).

In another study (DAR: Ben-Dyke, 1977b), bupirimate (95.5-97.0% purity) was given by dietary admixture at concentrations 0, 100 and 1000 ppm to mice (40/sex/dose) daily for 80 weeks. Body weight was measured weekly for the first 4 weeks and every 2 weeks thereafter and food consumption was measured weekly for the first 14 weeks and every 2 weeks thereafter. Water consumption was assessed visually. After 80 weeks all surviving animals were killed and necropsied. 10 male and 10 female animals in each group were subjected to histopathological evaluation of all collected tissues. Only a limited selection of tissues from all other animals (those suggestive of neoplasia, and adrenals, liver, lymph nodes, ovaries, pituitary, spleen and thyroids) was examined microscopically.

The average daily bupirimate intakes were calculated to be:

100 ppm: 10.0-18.4 mg/kg bw/day (males); 11.3-23.5 mg/kg bw/day (females)

1000 ppm: 113.0 – 177.4 mg/kg bw/day (males); 120.5-242.3 mg/kg bw/day (females)

At the end of the study survival was only 25-38% in females and 15-25% in males; survival in each group was ≥ 50% until the 60th week of the study. There were no clinical signs or treatment-related mortality. Compared to levels at the start and the end of the study, food intake showed a dip in the middle period in both bupirimate-treated groups in both sexes, which was strongest after half a year in 100 ppm treated females (food intake 11% less than control over a period of 12 weeks). Growth patterns of mice of both sexes receiving bupirimate at 100 and 1000 ppm were equivalent to the control. Macroscopic and microscopic examination revealed a normal range of spontaneous pathology affecting the urinogenital, gastrointestinal and reticulo endothelial systems, and the types and distribution of pathological lesions were not associated with the treatment. The neoplasms present showed no related treatment disturbance in their distribution among groups. There was no evidence of any treatment-related increase in the proportion of tumour-bearing mice.

The incidences of the different tumour types were generally within the range of normal expectation for mice of this age and strain. The distribution across the groups was random.

The study had the following deviations from OECD guideline 451: histopathological examination of tissues was only performed on 10 males and 10 females from the highest and control group, instead of all animals of these groups (except for 7 tissues, which were examined in all animals at all dose levels); number of animals used per group was 40 instead fo 50, number of dose groups was 2 instead of 3, and high mortality (up to 85% instead of max. 50%) was observed. The study was considered not acceptable.

Within the limitations of the study designs, there was no evidence that chronic dietary administration of bupirimate toe the CD-1 mice at concentrations up to 1000 ppm (~ 100 mg/kg bw/day) was associated with an increased risk of carcinogenicity.

In the third study (DAR: Ben-Dyke et al., 1977c) bupirimate (purity 95.5-99.5% was administered orally ingelatine capsules at dose levels of 0, 5, 20 and 200 mg/kg bw/day daily for 105 weeks to 5 groups of 4 male and 4 female Beagle dogs (20-25 weeks of age). All doses were administered about 3 hours after the 1st daily meal. Clinical observations were recorded daily and body weights weekly. Food residues were weighed and weekly consumption calculated. Ophthalmoscopic examinations were made before the start of treatment and in weeks 13, 26, 52, 78 and 104. Blood samples for haematological and biochemical evaluation were taken from each animal before the start of treatment and after 6, 13, 26, 52, 78 and 104 weeks. An additional blood sample was taken in week 104, 15 minutes after the intravenous injection (10 mg/kg bw) of a 5% solution of BSP for assessment of liver clearance. Overnight urine samples were collected under food and water deprivation before the start of treatment and during weeks 6, 13, 26, 78 and 104. At the end of the treatment period all animals were killed and necropsied. Organ weights were recorded and a selection of tissues from all animals was examined microscopically. In week 42 one control male died for reasons not associated with the study. An additional control male was included on the study in week 49.

Table 25. Results from 105 weeks oral toxicity study in dogs (DAR: Ben-Dyke et al., 1977c)

Dose (mg/kg bw/d)	0		5		20		200		dr
Sex	m	f	m	f	m	f	m	f	
Mortality	no toxicologically relevant effect								
Clinical signs									
emesis	0/4	0/4	0/4	0/4	0/4	0/4	4/4	1/4	
salivation	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	
Body weight (at 105 wk, Δ% of control)			-6		-12		-16		dr m
Body weight gain (0-105 wks, % of control-100)			-26		-43		-47[a]		dr m
Food consumption (0-105 wks, % of control-100)	-	-	-	-	-2	+6	-8	+6	dr
Ophthalmoscopy	no toxicologically relevant effect								
Haematology									
PVC				-		ds		ds	dr f
Hb				ds (wk 13)		ds		ds	dr f
RBC				-		ds		ds	dr f
Clinical Chemistry									
SAP					is	is	is	is	dr
ALAT					-	-	is	is	
Urinalysis	no toxicologically relevant effect								
Organ weights (% of control-100)									
liver weight (abs.)					+9	+12	+26	+27	dr
liver weight (rel.)			+11		+27*	+11	+43**	+26**	dr
thyroid weight (rel.)			+28	-26	+4	+14	+28	+21	dr f
Pathology									
Liver:									
Increased no. of pigmented macrophages	0/4	0/4	0/4	0/4	0/4	0/4	3/4	3/4	
Increased no. of pigmented Kupffer cells	0/4	0/4	0/4	0/4	0/4	1/4	1/4	2/4	
Fibrosis	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	
Cytoplasmic vacuolation	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	
Increased lipofuscin	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4	

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly
[a] statistically significant different from control in ANOVA ($p<0.05$) until 78 weeks.
* $p<0.05$; ** $P<0.01$; *** $p<0.001$ (compared with control group, test not specified)
Δ% of control = body weight after 105 weeks in % of control, minus bodyweight at 0 weeks as % of control

Bupirimate at dosage of 200 mg/kg bw/day was associated with emesis and salivation (see table 25). With the exception fo the period between weeks 9 and 23, males receiving 200 mg/kg bw/day

had reduced food intake (max. 14% less than controls over weeks 27-52). Females in the highest dosing group showed a decrease in food intake during the first 8 weeks of treatment but the overall food consumption in the 2 highest dosing groups was slightly higher than controls. Administration of bupirimate to male dogs was associated with a reduced weight gain, which was statistically significant at 200 mg/kg bw/day until the 78th week. In the low dose the decrease was only marginal and the range of body weights of the low dose animals was similar to that of the controls.

PCV, Hb and RBC concentration values were statistically significantly lower (mostly between -10 and -17% with respect to controls) in females receiving 20 and 200 mg/kg bw/day on several time points during treatment, and in females receiving 5 mg/kg bg/day only in week 13. Increases (up to 2-4 times) in serum alkaline phosphatise (20 and 200 mg/kg bw/day) and glutamate pyruvate transaminase activities (200 mg/kg bw/day) occurred in both male and female dogs. These changes showed some correlation with increases in liver weight and the presence of lipufuscin in some hepatocytes.

At necropsy, 3/8 animals receiving 200 mg/kg bw/day bupirimate had dark colouration of the liver. Body weight-related liver was increased in males and females receiving 20 and 200 mg/kg bw/day and in males only of the 5 mg/kg bw/day group. The body weight-related thyroid weight was increased in both sexes at 200 mg/kg bw/day, in females at 20 mg/kg bw/day, and in males of the 5 mg/kg bw/day group.

Histopathological examination revealed treatment-related changes in the liver. Increased numbers of pigment-laden macrophages in the portal tracts or pigment containing Kupffer cells in the hepatic sinusoids were noted in 6/8 animals receiving 200 mg/kg bw/day and 1/8 animals receiving 20 mg/kg bw/day. The appearance of the pigment was typical of lipofuscin and in 2 dogs receiving 200 mg/kg bw/day the generalised degree of hepatic lipofuscin pigmentation was greater than normal. In a single female receiving 200 mg/kg bw/day there was a significant degree of hepatocytic cytoplasmic vaculolation, and in a single male receiving 200 mg/kg bw/day fibrosis of the liver was observed.

The reduced body weight (gain) in treated male dogs appears to be a result of the relatively high body weight (gain) in control males, which were outside the somewhat concise historical control data. The effects on body weight were considered not treatment-related. This is consistent with the 90-day dog study, in which only at the high level (600 mg/kg bw/day) an adverse effect on body weight was observed in males and females, but not at 30 mg/kg bw/day and lower.

No adverse effects were observed in the thyroids of dogs in this 105 weeks study. No indications of carcinogenicity of bupirimate were found in this study, either.

The study resembled OECD guideline 452 and is considered acceptable.

Oral (capsule) administration to the beagle dog, daily for 105 weeks resulted in effects on liver weight, serum liver enzymes and liver pathology at 20 mg/kg bw/day. The effects observed at 5 mg/kg bw/day were not consistent over time (haematology), only marginal without concurrent findings in clinical chemistry/histopathology (liver weights) or not dose-related (thyroid weights). Hence the NOAEL was considered to be 5 mg/kg bw/day.

4.8.1.2 Carcinogenicity: inhalation

This information is not available.

4.8.1.3 Carcinogenicity: dermal

This information is not available.

4.8.2 Human information

No human information is available.

4.8.3 Other relevant information

This information is not available.

4.8.4 Summary and discussion of carcinogenicity

Three long-term oral toxicity studies were available: an 18-months carcinogenicity study in the mouse, a 2-year combined chronic toxicity/carcinogenicity study in the rat and a 2-year chronic toxicity study in the dog. In the mouse study no toxicity was seen, but this study was unacceptable due to poor survival, limited selection of tissues examined, small group sizes and presence of only 2 dosing levels.

In the 2-year study in the dog, no increase in tumour formation was observed.

In the rat study, treatment-related effects were observed at the highest dose of 5000 ppm: a decrease in body weight gain and relative liver and thyroid weight in both sexes and statistically significantly increased incidences of follicular adenomas in the thyroid (males only) and fibromas in the skin (females). Since bupirimate can be considered a non-genotoxic agent, the mechanism behind tumour formation in the rat is not genotoxicity.

As to thyroid tumour formation, it was demonstrated that bupirimate can induce prolonged disturbances in the hypothalamus – pituitary – thyroid (HPT) axis, as shown by altered circulating hormone levels and increased thyroid weight at dose levels similar to the dose inducing thyroid tumours in rats (see Ashby, 1979 in chapter 4.10.1.3). It is known that hyperactivity of the HPT-axis can lead to thyroid follicular cell tumours in rats (especially in male rats). The thyroid effects can be linked to a modified metabolism of a thyroid hormone (thyroxine T4) with a release of increased amounts of TSH to restore homeostatic conditions. Treatments initiating thyroid-pituitary disruption in rodents result in chronic reduction in circulating thyroid hormone levels, increase in TSH levels and the development of increased cell division, increased size and numbers of thyroid cells, increased thyroid gland weight and in some cases tumours of the thyroid. This activation is a well-known phenomenon occurring particularly in rats because thyroxine metabolism takes place very rapidly in rats while humans have a substantial reserve supply of thyroid hormone, much of which carried in thyroxine-binding globulin, a serum protein that is missing in laboratory rodents. The results are consistent with the hypothesis that the promotion of thyroid tumours in rats was not a direct effect of bupirimate on the thyroid gland, but rather an indirect effect mediated by TSH secretion from the pituitary secondary to the hepatic microsomal enzyme-induced increase of T4 excretion in the bile. However, in the available studies the direct evidence for this mode of action, e.g. clearly increased TSH-secretion and hepatic microsomal enzyme-induced decrease of T4 in blood is rather limited. However, there are no indications of thyroid tumours in mice, dogs or female rats, although the liver weights are affected. Therefore, the mechanism causing thyroid tumours in the rat might be species specific. However, for a definitive conclusion, more data are needed.

Humans are considerably less sensitive to disturbances in the HPT-axis and subsequently to the development of these thyroid follicular cell tumours. Hence in the absence of genotoxic properties of bupirimate, thyroid tumours in rats induced by this substance by disturbance of the HPT-axis are considered not relevant for human risk assessment. Moreover, in the report of the EU committee of specialised experts in the fields of carcinogenicity, mutagenicity and reprotoxicity, meeting at Arona 1-2 September 1999, it is agreed by specialized experts that non-genotoxic carcinogenic substances producing thyroid tumours in rodents with low/medium potency by a clearly established perturbation of the thyroid hormone axis do not need to be classified. This interpretation is included in the present ECHA guidance on the Application of CLP criteria (3.6.2.3.1).

Skin fibroma

The incidence of skin fibroma was statistically significant increased in female rats at the highest dose level and outside the range of the historical controls. In males a non-significant increase was observed which was within the range of the historical controls.

4.8.5 Comparison with criteria

The increase in mammary adenocarcinoma in female rats does not warrant classification because it is not statistically significant and within the range of the historical control data. The increase in thyroid follicular adenoma is significant. However, the adenoma is most likely induced through a mechanism less relevant to humans. In line with the criteria of the specialised experts no classification due to these adenoma's is proposed.

Skin fibroma

In the DAR the following conclusion was drawn:

The incidence of skin fibroma for females was just outside the historical control data, but the historical control data for the Sprague-Dawley rat from the performing laboratory and the supplier of the rat strain suggest that skin fibroma is a common, spontaneous tumour found in the aging rat. This taken together with the lack of evidence for carcinogenicity or genotoxicity from all other studies suggests that bupirimate dose not pose a carcinogenic hazard to man and that a classification for carcinogenicity is not required.

During the EFSA discussions on bupirimate for carcinogenicity, the notifier provided a position statement (Makhteshim Chemical Works Ltd., 2006) concluding that the weight and strength of the evidence of bupirimate and its main metabolite ethirimol suggests that classification is not required. The position paper is added to the IUCLID as an attachment. Additional arguments in this position paper were that skin fibroma were regularly observed in male (1-11%) and female (1-4%) rats in a non-contemporary historical control database and that no comparable increase was observed in a rat carcinogenicity study with ethirimol, the main metabolite of bupirimate.

The current assessors do not fully agree with the conclusion in the DAR and of the notifier. The increase in skin fibroma in female rats was statistically significant and outside the historical control range. The results of the carcinogenicity study with ethirimol, the main metabolite of bupirimate,

did not show an increase in skin fibroma. However, this study was performed with a top dose level of 500 ppm whereas the carcinogenicity study with bupirimate used 5000 ppm as the top dose level. At 500 ppm with bupirimate also no increase in skin fibroma was observed. The results with ethirimol are therefore no justification that the increase in skin fibroma with bupirimate at 5000 ppm are a chance finding. Also, it cannot be excluded that the other main metabolite ethyl-guanidine, which is not formed from ethirimol, has a role in the increase of skin fibroma. Further, an increase of skin fibroma's was observed in both males and females compared to the historical controls, although the increase in males was not statistically significant and within the historical control range. Combining the incidence of males and females in the study and comparing this with the combined incidence in the historical controls is not an acceptable method because this could result in incorrect assessment of tumours induced through a sex specific mechanism. The absence of similar tumours in mice and dogs is not shown as in the mice study only for 10 mice per sex a full investigation of all tissues was performed whereas for the remaining mice only a limited number of tissues was examined not including skin but including those suggestive of neoplasia. This reduces the likelihood of detecting skin tumours. This and other limitations resulted in a conclusion that the study was not acceptable. The available 2-year study in dogs is not designed for detection of tumours as it uses only 4 dogs per sex. No dose-effect relation was observed for skin fibroma's in the rat study with bupirimate. However, the dose spacing was a factor of 50 between the lowest and highest dose. This is more than usual in carcinogenicity studies and makes finding a dose response relation difficult for a threshold effect. There is no information on the mechanism of these tumours. Therefore, the relevance of these tumours for humans cannot be excluded.

Overall, there is a weak but significant increase in female skin fibromas outside the range of the historical controls. The increase is only small making it not a strong case for classification but there is no indication that this type of tumour is not relevant to humans. With these animal data, classification as a carcinogenic substance is justified. As the carcinogenic effect was only observed in one species and one sex and bupirimate is not genotoxic, the strength of evidence is limited. Thus a classification as Carc cat 3, R40 (DSD) and Carc 2, H351 (CLP) is proposed.

4.8.6 Conclusions on classification and labelling

Table 26 Conclusion on classification for carcinogenicity

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc 2; H351 (all routes)	Carc Cat 3; R40

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. Skin fibromas are considered as common (benign) lesions in aging rats.	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 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%
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 9-12 studies	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
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.

4.9 Toxicity for reproduction

Table 27: Summary table of relevant reproductive toxicity studies

Method	Test details	Results (NOAEL/LOAEL)	Study Quality (Klimisch score)	Reference
Multi-generation oral diet study, rat Resembled OECD 416 No GLP	15 m + 25 f / group Dose: 0, 100, 400, and 4000 ppm	$NOAEL_{parental}$ = 20 mg/kg bw/day (400 ppm) $NOAEL_{offspring}$ = 20 mg/kg bw/day (400 ppm) $NOAEL_{reproductive}$ = 200 mg/kg bw/day (4000 ppm) $LOAEL_{parental}$ = 200 mg/kg bw/day (4000 ppm) $LOAEL_{offspring}$ = 200 mg/kg bw/day (4000 ppm) $LOAEL_{reproductive}$: not established Relative liver and kidney weight ↑ (weaned progeny), bodyweight ↓ (parent and offspring), delay in physical development (offspring)	Reliable with restrictions	DAR (Tesh et al., 1977)
Developmental oral gavage, rat, OECD 414 GLP	15 m + 25 f / group Dose: 0, 50, 150 and 400 mg/kg bw/d Daily dosing at gestation days 7-16	$NOAEL_{maternal}$ < 50 mg/kg bw/day; $NOAEL_{development}$ = 50 mg/kg b/wday $LOAEL_{maternal}$ = 50 mg/kg bw/day $LOAEL_{development}$ = 150 mg/kg bw/day Marginal clinical signs ↑, body weight gain ↓ (maternal), minor skeletal defects (developmental)	Reliable without restrictions	DAR (Moxon, 1992)
Teratology, oral, gavage, rabbit Resembled OECD 414 No GLP	10-11 f / groupDose: 0, 50, 100 and 500 mg/kg bw/d Dosing on days 1-28 of gestation	No conclusions drawn because of lack of reliability	Not reliable	DAR (Hodge and Palmer, 1975)
Developmental oral gavage, rabbit, OECD 414 GLP	22 f/groupDose: 0, 20, 80 and 320 mg/kg bw/d Dosing on gestation days 6-28	$NOAEL_{maternal}$ = 20 mg/kg bw/day $NOAEL_{development}$ = 80 mg/kg bw/day $LOAEL_{maternal}$ = 80 mg/kg bw/day $LOAEL_{development}$ = 320 mg/kg bw/day Body weight gain ↓, food consumption ↓, abortions ↑ (maternal), unossified skeletal elements ↑, supernumerary ribs ↑ (developmental)	Reliable without restrictions	DAR (Chevalier, 2006)

4.9.1 Effects on fertility

4.9.1.1 Non-human information

Bupirimate (purity 95.5-99.5%) was given by dietary admixture at concentrations of 0, 100, 400 and 4000 ppm to groups of 15 male and 25 female CD rats continuously throughout 3 generations (F0, F1 and F2) with 2 litters (A and B) per generation (DAR: Tesh et al., 1977). The premating period in each generation was approximately 70 days and a 10-day rest period was allowed between the end of weaning of the first litters and pairing for mating to produce the second litters.

The observations described below were conducted for each of the 3 generations. Parental animals were observed daily and their body weight was measured weekly except for mated females which were weighed on gestation days (GD) 1, 3, 7, 14 and 21. Parental animal food consumption was only recorded during the pre-mating period. Oestrus cycles were monitored. For mating, females were paired 2:1 with males and allowed 5 days to mate. Non-mated female were re-paired with a different male for a further 5 days. This was repeated for up to 2 more times so that each female had up to 20 days to mate. Once mater the females were separated. The second mating period commenced approximately 10 days after weaning the first litters. Different pairs were mated at each mating phase in each generation and sibling parings were avoided.

Not all parental females were allowed to litter. After each mating, 5 randomly selected females from each groups were killed on GD 13 and maternal condition, the number of corpora lutea in each ovary, the number, viability and distribution of embryos in each uterine horn and the number of resorption sites were recorded. Pre- and post-implantation losses were calculated.

The remaining females delivered their young to provide information on gestation length, parturition, litter size, birth-weight, viability and incidence of abnormal offspring. The viability and development of the offspring were assessed, and litters were weighed on postnatal days (PND) 1, 4, 10 and 21. Physical development (pinna unfolding, hair growth, tooth eruption and eye opening) of the offspring was monitored during lactation. Auditory and visual functions were assessed at weaning.

From the F1b and F2b litters, 15 males and 25 females per group were selected to provide the next generation, all other pups were killed after weaning. The parental animals were killed after the second weaning phase in each generation and together with all surplus weaned offspring, were subject to gross necropsy for external and internal abnormalities. Additionally, for the F3b litters, selected organs and tissues from 20 randomly selected male and 20 female weanlings per group were weighed and subjected to histopathological evaluation, and of the number of organs (13 in total) the weight was taken.

The range of average daily bupirimate intakes during the maturation periods only were calculated to be:

Parental males, 100 ppm: 5-11 mg/kg bw/day (F0 generation), 6-12 mg/kg bw/day (F1 generation), 6-12 mg/kg bw/day (F2 generation) (overall 5-12 mg/kg bw/day).

Parental males, 400 ppm: 21-45 mg/kg bw/day (F0 generation), 22-56 mg/kg bw/day (F1 generation), 236-560 mg/kg bw/day (F2 generation) (overall 201-560 mg/kg bw/day)

Parental males, 4000 ppm: 201-462 mg/kg bw/day (F0 generation), 252-472 mg/kg bw/day (F1 generation), 236-560 mg/kg bw/day (F2 generation) (overall 201-560 mg/kg bw/day)

Parental females, 100 ppm: 7-11 mg/kg bw/day (F0 generation), 8-12 mg/kg bw/day (F1 generation), 8-14 mg/kg bw/day (F2 generation) (overall 7-14 mg/kg bw/day)

Parental females, 400 ppm: 26-45 mg/kg bw/day (F0 generation), 34-51 mg/kg bw/day (F1 generation), 31-52 mg/kg bw/day (F2 generation) (overall 26-52 mg/kg bw/day)

Parental females, 4000 ppm: 296-496 mg/kg bw/day (F0 generation), 321-477 mg/kg bw/day (F1 generation), 316-604 mg/kg bw/day (F2 generation) (overall 296-604 mg/kg bw/day).

Table 28. Results from a multigeneration study in the rat.

	Dose (ppm)	0		100		400		4000		dr
	Sex	m	f	m	f	m	f	m	f	
F0 animals	mortality	no toxicologically relevant effect								
	clinical signs ungroomed appearance				i[1]		i[1]		i[1]	
	body weight (wk 24/10 m/f) (% of control -100)					-6	-4	-15**	-14**	
	food consumption	no toxicologically relevant effect								
	food conv. ratio (0-10 wk)	5.2	10.1	5.2	10.3	5.7	10.6	6.6**	13.6*	dr
	mating time	no toxicologically relevant effect								
(1st / 2nd litter)	fertility (pregnant / paired) non-pregnant females (#)	4 / 6 (2)		6 / 7 (3)		i / i 2 / 1[a] (1)		i / i 1 / 0 (0)		
	gestation length	no toxicologically relevant effect								
	oestrus cycle	no toxicologically relevant effect								
	pre implantation loss (%) post implantation loss (%)		9 / 3 5 / 5		14 / 16 4 / 3		5 / 14 11 / 9		8 / 15 7 / 9	
F1 pups	litter size	no toxicologically relevant effect								
	survival index							- / d		
(1st / 2nd litter)	body weight (PND 25) (% of control -100)					+11 / - 2		-7* / -9*		
	development tooth eruption time eye-opening time							- / i i / -		
	sex ratio	no toxicologically relevant effect								
F1 animals	mortality	no toxicologically relevant effect								
	clinical signs	no toxicologically relevant effect								
	body weight (wk 23/10 m/f) (% of control -100)			+12*	+5	+3	-1	-9*	-15**	dr
	food consumption food conv. ratio (0-10 wk)								d i	
	mating time	no toxicologically relevant effect								
(1st / 2nd litter)	fertility non-pregnant females (#)	5 / 0 (0)		- / d 4 / 5 (2)		i / - 2 / 1 (0)		i / - 1 / 2 (0)		
	gestation length	no toxicologically relevant effect								
	oestrus cycle	no toxicologically relevant effect								
	pre implantation loss (%) post implantation loss (%)		8 / 5 2 / 1	-	37 / 10 0 / 4		13 / 8 0 / 5		18 / 7 6 / 7	
F2 pups	litter size			ds* /				- / d		
	survival index							- / ds*		
(1st / 2nd litter)	body weight (PND 25) (% of control -100)							-13** / -0.5		
	development eye opening time							i / -		
	sex ratio	no toxicologically relevant effect								
F2 animals	mortality	no toxicologically relevant effect								
	clinical signs	no toxicologically relevant effect								
	body weight (wk 24/10 m/f) (% of control -100)			+11**	+13**	0	0	-13**	-12**	dr
	food consumption	no toxicologically relevant effect								
(1st / 2nd litter)	food conv. ratio (0-10 wk)	no toxicologically relevant effect								
	mating time			is**/ is*						
	fertility (pregnants/paired) non-pregnant females (#)	2 / 2 (0)		d / d 8 / 5 (3)		i / - 0 / 1 (0)		i / - 0 / 1 (0)		
	gestation length	no toxicologically relevant effect								
	oestrus cycle	no toxicologically relevant effect								

	Dose (ppm)	0		100		400		4000		dr
	Sex	m	f	m	f	m	f	m	f	
	pre implantation loss (%) post implantation loss (%)		6 / 5 0 / 3		38 / 2 2 / 5		10 / 3 4 / 4		8 / 12 0 / 26	
F3 pups (1st / 2nd litter)	litter size			ds* / -		- / i				
	survival index			- / i		- / i		- / i		
	body weight (PND 25) (% of control -100)			+6 / +6		-3 / -9		-19** / -17**		dr
	development eye opening time hair growth time pinna unfolding					i / i i / -		i / i i / - - / i		 dr dr
	sex ratio	no toxicologically relevant effect								
	organ weight (% of control-100) rel. liver rel. kidney rel. thyroid			+8* +5*	+6 -2	+12** +6*	+6 0	+31*** +10*** i	+23*** +6 i	 dr m dr m
	pathology - microscopy / macroscopy	no toxicologically relevant effect								

dr = dose related; i = increased; d = decreased; is = increased significantly; ds = decreased significantly; PND = post natal day
[1] only during gestation and lactation of first litters
(#) Figure in brackets is the amount of females that were both times not pregnant.
[a] Figure is derived of a group with one female less than in the other groups (i.e. 19 instead of 20 females for second litter)

Parental animals in all generations showed no treatment-related changes in clinical condition apart from an ungroomed appearance during gestation and lactation of first litters in all bupirimate dosed females of the F0-generation. Administration of 4000 ppm bupirimate was associated with lower body weight gains in parental males and females, and also in pups during lactation, throughout all generations. In the F3b litters, the 400 ppm pups showed decreased weight gain compared to the concurrent control pups, but the difference in weight gain in this dose group is attributable to both the higher mean number of pups per litter at birth (12.0 in the 400 ppm group compared to 9.0 in the controls) and to markedly lower pup mortality (1.2 mean percent mortality at post-natal day (PND 4) in the 400 ppm group compared to 22.5 mean percent mortality at PND 4 in the controls). There were thus more surviving pups per litter (litters were not standardized or culled) competing for nutrition in the 400 ppm group compared to the control group.

Opposite effects were measured on fertility (numbers of animals mating and conception rats), as fertility seemed decreased in animals dosed 100 ppm, but increased in animals dosed 400 and 4000 ppm. Mean numbers of corpora lutea were unaffected by treatment in the small subgroup sacrificed on GD13 in each generation, but number of mean viable young per pregnant rat was decreased in all three groups of dosed animals. However, no effects were seen on litter size of females allowed to litter. Pup survival in the second litters of the first 2 generations was lower at 4000 ppm; generally a consequence of total litter loss in one or two females. The physical development of the pups was slightly retarded at 4000 ppm, as evidenced by occasional delays in eye opening, tooth eruption, hair growth and pinna unfolding. There was no effect on auditory or visual functions. At 400 ppm there were no consistent effects on developmental parameters. In the 400 ppm F3b pups, slight developmental delays compared to the concurrent F3b controls were noted, however, these delays correlate with the reduced weight gain compared to the F3b control group. As noted above, the assessment of weight gain in this generation is skewed by the high control pup mortality, resulting in fewer pups per litter and consequently faster control pup growth. Comparison with F1b and F2b control data shows the F3b developmental parameters at 400 ppm fall within a normal control range.

Liver and kidney weights (relative to body weight0 of the F3b offspring were increased at all three dose levels in males, and only in the highest dose level in females. Absolute kidney weights, however, were lower in females dosed 400 and 4000 ppm, and in males dosed 4000 ppm bupirimate. Moreover, as only in the highest dose group an adverse effect on body weight was

observed, the relative organ weights in the low and mid dose group are less relevant. Relative thyroid weight seemed slightly increased in highest dose groups. In the highest dose group differences from the controls in weight of other organs than liver, kidney and thyrolid were considered to be a consequence of differences in body weight, not related to treatment. No treatment related histopathological findings were seen at any dose level in the F3b pups evaluated. There were no reported treatment-related gross necropsy findings in the parental animals or the surplus weaned pups.

The study resembled OECD Guideline 416 and is considered acceptable.

Based on the results of the study, bupirimate was considered not reproductive toxic in the tested concentrations; the NOAEL for reprotoxicity was 4000 ppm (equal to 200 mg/kg bw/day). Bupirimate showed no evidence that pups were more sensitive than adults. Based on the intreased organ weight of liver and kidney in weaned progeny, delayed physical development in pups and decreased body weights at 4000 ppm, the NOAEL for both offspring and parental effects was 400 ppm (equal to 20 mg/kg bw/day).

4.9.1.2 Human information

No human information is available.

4.9.2 Developmental toxicity

4.9.2.1 Non-human information

In the study of Moxon (DAR: 1992), four groups of 24 mated female Wistar derived Alpk:APfSD rats received bupirimate (purity 95.6%) by oral gavage as a suspension in corn oil at dose levels of 0, 50, 150 or 400 mg/kg bw/day from gestation day (GD) 7 to 16, inclusive. The control group received corn oil alone. The animals were observed daily. Body weight was recorded on GD 1, 4, 7-16 (inclusive), 19 and 22. Food consumption was measured over 3 day periods.

On GD22 the females were killed, a macroscopic necropsy was conduced and pregnancy status was determined. The overaies were removaed and examined and the gravid uterus removed and weighed. Number of corpora lutea and numbers of live foetuses and early or late resorption sites were recorded. After weighing, the foetuses were killed and each foetus was examined for external abnormalities and visceral abnormalities. Foetuses were then eviscerated and fixed in methanol. The skull was cut along the fronto-parietal suture and the brain examined. The carcasses were returned to methanol for subsequent processing and staining with Alizarin Red S. The stained foetal skeletons were examined for abnormalities and the degree of ossification assessed.

The observations were classified as major (permanent structural or functional deviations considered likely to be incompatible with survival or rarely seen) or minor defects or variants (small, generally transient deviations considered compatible with survival).

Table 29. Results of developmental toxicity study with bupirimate in rats

dose (mg/kg bw/d)	0	50	150	400	dose related
Maternal toxicity					
Mortality	no toxicologically relevant effect				
Clinical signs - salivation - urinary incontinence	 0/22 0/22	 1/22 2/22	 3/23 10/23	 22/22 21/22	 dr dr
Body weight gain		d	d	ds	dr
Food consumption				ds	
Gross pathology	no toxicologically relevant effect				
Pregnancies	22/24	22/24	23/24	22/24	
Uterus weight	no toxicologically relevant effect				
No. corpora lutea	no toxicologically relevant effect				
No. implantations	no toxicologically relevant effect				
Intra-uterine deaths.	no toxicologically relevant effect				
Post implantation loss	no toxicologically relevant effect				
Sex distr. (% males)	56.2	54.0	57.3	43.4 (ds)	
Foetal toxicity					
Body weights	no toxicologically relevant effect				
Mean litter weight	no toxicologically relevant effect				
No. of live foetuses	no toxicologically relevant effect				
Malformations - external - visceral	 no toxicologically relevant effect no toxicologically relevant effect				
- minor skeletal defects only (%)	29.2	28.6	39.2	41.1 (is)	dr

i/d=increased/decreased, is/ds=significantly increased/decreased, a/r=absolute/relative, dr=dose related.

There were no maternal deaths during the study. Administration of bupirimate was associated with salivation and/or signs of urinary incontinence. Also at the lowest dose level (50 mg/kg bw/day) 3 animals were affected. Administration of 300 mg/kg bw/day was associated with a lower maternal body weight gain; this persisted after the cessation of treatment. A marginal decrease in body weight gain was also apparent at 50 and 150 mg/kg bw/day, but only during the treatment period (GD 8-16). Food consumption at 400 mg/kg bw/day was reduced (10-15%) throughout the dosing period. There was no evidence of any adverse effect of bupirimate on the number, growth or survival of the foetuses in utero. Pre-implantation loss was higher at 400 mg/kg bw/day than in the controls, but, as implantation occurs prior to the start of the dosing period, this was considered unrelated to bupirimate treatment. There was a significantly lower number of male foetuses per litter at 400 mg/kg bw/dayl this was also considered unrelated to treatment in the absence of increases in embryonic deaths (post-implantation losses0. There were no treatment-related effects on foetal body weight. The 7^{th} cervical and 4^{th} lumbar transverse processes were less well ossified in some foetuses from the 150 and 300 mg/kg bw/day groups. No effect of exposure to the test substance was noted on the individual bones of the manus and pes.

The study is conducted according to OECD Guideline 414 and is considered acceptable.

Maternal toxicity was evident at the two highest dose groups, although in a few animals of the low dose group clinical signs were also observed. Since these clinical signs, and the decrease in body weight gain, are considered to be treatment-related, the marginal LOAEL for maternal toxicity in this study is 50 mg/kg bw/day. Minor skeletal defects were seen in the two highest dose groups; the NOAEL for foetotoxicity is 50 mg/kg bw/day. The test substance did not induce structural irreversible effects.

In the study of Hodge and Palmer (DAR: 1975) 48 female Dutch rabbits were mated with 6 male Dutch rabbits. One hour after mating, each dose received 25 iu of chorionic gonadotrophin in order to promote ovulation. The day of mating was day 0 of gestational period, and animals were dosed from day 1 up to and including day 28. Doses were 0, 50, 100 and 500 mg/kg bw/day in Tween 80.

Controls received 0.5% Tween 80 alone. The does were observed daily for clinical signs and body weights were recorded every 4 days. On day 29 the does were killed, the foetuses were removed by Caesarian section and the uterus examined for resorptions. The corpora lutea were counted and compared with the number of implantation sites. Abnormal foetuses and maternal organs were examined histologically. After Caesarian section, maternal tissue were routinely examined. Only if the maternal heath status was in doubt, tissues were examined histologically. Foetuses were assessed for viability, weighed and killed. They were sexed and external abnormalities were noted. One half of the foetuses of each litter was eviscerated, skinned, fixed in alcohol, and stained for subsequent skeletal examination. The remaining foetuses were fixed and decalcified before visceral examination.

Three animals (one control animal, one animal treated at 50 mg/kg bw/day and one treated at 500 mg/kg bw/day) were excluded due to illness, death, or early delivery. Two other animals (one control and one treated at 100 mg/kg bw/day) did not litter. Hence at the end of the study only 10 control females and 11 females per treated group produced litter.

Maternal toxicity was not observed, although it should be mentioned that the body weight increase of the pregnant does was very low. The reported sex ratio (m/f) in the control group of 0.67 is considered low. The number of live foetuses and mean number of foetuses per litter were dose-relatedly decreased, but in the lowest 2 dose groups this was mainly explained by the already lower number of corpora lutea (absolute or per litter, as could be calculated from the implantation index). Effects on number of corpora lutea are coincidental, as dosing started only after conception. In the highest dose group the number of foetuses per litter was decreased due to a combination of a lower implantation index and a statistically significantly increased percentage of early resorptions. Lower mean litter weights were consistent with the lower amount of foetuses per litter in all dose groups. Skeletal deviations were dose relatedly increased in some bones, but findings were not consistent, as in other bones, controls were more affected.

The study resembled OECD guideline 414, with the following deviations; there were too few pregnant females at necropsy (10-11 instead of minimally 16); body weight was recorded every 4 days instead of at least every 3 days during the dosing period; food consumption was not recorded; uterine weight was not determined; no data were provided on visceral examination fo the foetuses, and no data were provided on abortions and dead foetuses.

The authors stated that the observations in the dose groups were within normal control limits; however, historical control data werenot provided. The study is considered not acceptable.

Chevalier (DAR: 2006) administered bupirimate by daily oral administration at 20, 80 or 320 mg/kg bw/day to groups of 22 mated female KBL New Zealand White rabbits from GD 6 to GD 28. The study was conducted according to OECD guideline 414. 22 females received the vehicle alone (0.5% methylcellulose). Clinical signs and mortality were checked daily. Body weight and food consumption were recorded at designated intervals. On GD 29 the females were sacrificed and subjected to a macroscopic post-mortem examination of the principal organs. The gravid uterus was weighed to allow calculation of the net body weight gain and the placenta was also weighed. The fetuses were removed by caesarean section. The following litter parameters were recorded; number of corpora lutea, implantation sites, early and late resorptions, dead and live fetuses. The fetuses were weighed and subjected to external, soft tissue and skeletal (bone and cartilage) examinations.

Table 30. Results from teratogenicity study in the rabbit

	Dose (mg/kg bw/d)	0	20	80	320	dr
Maternal effects	mortality	3/22	2/22	1/22	0/22	
	clinical signs - vaginal discharge, blood in bedding	0/22	0/22	0/22	1/22	
	abortions	0/22	0/22	1/22	2/22	
	pregnant at ceasarian section	18/22	20/22	20/22	20/22	
	gravid uterine weight	No treatment-related effect				
	corpus lutea	No treatment-related effect				
	body weight (gain)a				dc	
	food consumption				dc (-54%)	
	water consumption	Not determined				
	pathology	No treatment-related effect				
Litter response	no. of implantations per litter	9.7	10.2	9.5	9.4	
	early resorptions (%)	2.9	2.0	2.1	4.2	
	late resorptions (%)	0.0	1.5	0.5	1.6	
	live foetuses	166	188	177	174	
	no. of foetuses per litter	9.4	9.9	9.3	8.9	
	foetal weight	No treatment-related effect				
	litter weight (g)	No treatment-related effect				
	sex ratio (m/f)	No treatment-related effect				
Foetus examination	no. of dead foetuses	No treatment-related effect				
	external malformations in fetuses (litters)	2/169 (2/18)	1/19 (1/20)	0/185 (0/20)	3/178 (3/20)	
	visceral malformations in fetuses (litters)	2/166 (2/18)	2/188 (2/20)	1/177 (1/20)	1/174 (1/20)	
	skeletal malformations in fetuses (litters)	2/166 (2/18)	4/188 (3/20)	4/177 (4/20)	11/174* (6/20)	
	skeletal deviations (%)					
	- Unossified hyoid centrum	0.6	2.1	3.4	7.5**	
	- Unossified first metacarpal	7.8	3.7	7.3	24.7#	
	- Incomplete ossification of talus	0.6	1.6	1.1	5.2*	
	- Unossified talus	0	1.1	1.7	5.7**	
	- Incomplete ossification of pubis	0	2.7	4.0 *	4.0*	
	- Full supernumerary ribs	53.6	57.4	49.2	78.2#	

*: p<0.05, **: p<0.01, #: p<0.001.

One female at 80 mg/kg bw/day and two females at 320 mg/kg bw/day aborted close to the end of the gestation period. At 320 mg/kg bw/day a body weight loss was noted from GD 6 to GD 15 and was associated with a low food consumption until GD 19. The abortions at 320 mg/kg bw/day were considered to be as a result of maternal toxicity. At 20 or 80 mg/kg bw/day the general conditions of females was unaffected by treatment.

No treatment-related effect on litter response was observed.

At 320 mg/kg bw/day the apparently higher number of malformed fetuses was due to one litter with 5 fetuses presenting eight lumbar vertebrae (instead of 7). This observation was limited to 1 litter only, is spontaneously found in control rabbits and was therefore considered to be not treatment-related. All other malformations did not show a dose-relationship and were considered to be spontaneous in origin, affecting one single fetus per group, or they were within the range of this laboratories historical control data. A higher incidence of incomplete ossification or non-ossification of the hyoid centrum, metacarpal, talus and pubis was noted at 320 mg/kg bw/day in comparison with the control group. Most of the incidences were statistically significant using the foetus as the unit. However, when the litter and the mean number of foetuses per litter were used as the unit, these observations were considered to be at their limit of biological significance. But an unequivocal relationship to treatment with the test substance could not be ruled out.

At 320 mg/kg bw/day slightly impaired ossification was noted in a few bones and the incidence of 13 ribs was slightly higher than in the control group but overall skeletal development was not affected by treatment.

The study is considered acceptable.

Under the conditions of the study, the NOAEL for maternal toxicity was 20 mg/kg bw/day. The NOAEL for developmental toxicity was 80 mg/kg bw/day.

4.9.2.2 Human information

No human information is available.

4.9.3 Other relevant information

This information is not available.

4.9.4 Summary and discussion of reproductive toxicity

In a three generation reproductive toxicity study, dietary administration of bupirimate at a dose level of 400 ppm (± 200 mg/kg bw/day) is associated with adverse effects on parental and offspring body weight. Furthermore, at 4000 ppm, physical development of pups is delayed and relative liver and kidney weights (investigated in F3b offspring only) are increased, without histopathological correlates. The NOAEL for offspring toxicity and for parental effects is 400 ppm (equal to ± 20 mg/kg bw/day). There is no evidence of adverse effects on reproduction (including mating performance, fertility or fecundity), therefore the $NOAEL_{reproduction}$ is 4000 ppm (equal to 200 mg/kg bw/day). In a rat developmental toxicity study, maternal toxicity, as evidenced by clinical signs (urinary incontinence) and retardation of body weight gain, is elicited by bupirimate during pregnancy in the rat at gavage doses of and greater than 150 mg/kg bw/day. As these effects were aso observed in a few animals of the 50 mg/kg bw/day dose group, the LOAEL for maternal toxicity is 50 mg/kg bw/day. Minor skeletal defects were seen in the 2 highest dose groups of 150 and 400 mg/kg bw/day, and therefore the NOAEL for developmental toxicity in this study is 50 mg/kg bw/day. The test substance did not induce structural irreversible effects.

In a rabbit developmental toxicity study, maternal toxicity (decreased body weight, food consumption and abortions) were observed in several animals at 320 mg/kg bw/day, and in one animal at 80 mg/kg bw/day. Hence the maternal NOAEL is set at 20 mg/kg bw/day. Litter parameters were not affected by treatment. At 320 mg/kg bw/day, a slightly higher incidence unossified skeletal elements and of supernumerary ribs was recorded; the developmental NOAEL is therefore set at 80 mg/kg bw/day.

4.9.5 Comparison with criteria

No signs of reproductive toxicity were observed in the 3-generation study with rats up to the highest dose level of 4000 ppm (ca. 200 mg/kg bw/day). In a developmental study with rats, minor skeletal effects were seen at dose levels eliciting maternal toxicity (manifested as lower maternal body weight gain and urinary incontinence). In a developmental study with rabbits, slightly higher incidence of unossified skeletal elements and of supernumerary ribs was recorded only at the highest dose level, at which maternal toxicity (decreased body weight, food consumption and abortions) was observed. Based on the results of the study, the substance does not need to be classified as a reproductive or developmental toxicant according to the EU classification criteria.

4.9.6 Conclusions on classification and labelling

Table 31. Conclusion on classification for reproductive and developmental toxicity

	CLP Regulation	Directive 67/548/EEC (DSD)
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Not classified	Not classified

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.

4.10 Other effects

4.10.1 Non-human information

4.10.1.1 Neurotoxicity

This information is not available. Since the substance is not a neurotoxin, specific neurotoxicity studies were not considered necessary. Clinical signs indicative of neurotoxic effects were not reported in the available repeated dose toxicity studies with rats, mice and dogs. Additionally, routine histopathological examinations of the brain, spinal cord and peripheral nerves did not detect any relevant abnormalitieis.

4.10.1.2 Immunotoxicity

This information is not available.

4.10.1.3 Specific investigations: other studies

Table 32 Characteristics

Reference/notifier	:	Ashby *et al.* (1979)	Exposure	:	daily for 28 days
Type of study	:	thyroid function	Doses	:	0, 5000 and 20,000 ppm
Year of execution	:	1979	Vehicle	:	0.5% aqueous gum tragacanth and 0.5% Tween 80 (1:1 v/v)
Test substance	:	bupirimate (purity 93.7%)	GLP statement	:	no
Route	:	oral; dietary intake	Guideline	:	none, study before OECD Guidance
Species	:	rat (Sprague Dawley, CD)	Acceptability	:	acceptable
Group size	:	30 m / dose	NOAEL	:	< 5000 ppm (equal to < 450 mg/kg bw/d) for effects on the thyroid

Study design

Bupirimate (purity 93.7%) was administered by dietary admixture at concentrations of 0, 5000 and 20,000 ppm, and by oral gavage at dose levels of 469 - 625 mg/kg bw/d (calculated to be equivalent to the achieved intake of the group receiving 5000 ppm in the diet; DAR: Asby et al., 1979). The oral gavage formulation was prepared in a combination of 0.5% aqueous gum tragacanth and 0.5% Tween 80 (1:1 v/v), and delivered at a constant dose volume of 10 mL/kg body weight. The homogeneity and stability of the dietary mixtures was assessed, and the achieved concentration of diet and liquid preparations was measured weekly. Groups of 30 five-week old male Sprague Dawley derived rats (CD, 92-150 g) were assigned to 4 treatment groups. They were housed in groups of 5 and tap water and a complete powdered rodent diet were freely available. Animals received bupirimate via the diet, or by oral gavage for up to 4 weeks. Clinical observations were made daily and body weight and food consumption was measured weekly.

After 2 weeks of treatment 5 animals from each group were killed, a further 10 were killed after 4 weeks. The remaining 15 animals were killed in groups of 5 at intervals of 6, 24 and 48 hours after receiving a single intravenous injection of I^{125} (sodium iodide solution BP 1.59 μCi/animal) upon completion of 4 weeks treatment. During this period the animals were maintained untreated. Thyroid stimulating hormone (TSH) production, thyroxin (T4) and triiodothyronine (T3) levels were examined after 2 and 4 weeks of treatment by radio-immunoassay. Ten animals per group were subject to a limited necropsy at the end of the 4-week treatment period. The animals were killed by exsanguination under ether anaesthesia and the thyroids were removed. The fixed thyroids were weighed and examined microscopically.

Body weight, clinical pathology and organ weight data were assessed by analysis of variance.

Results

All test diets and formulations used in the study were within acceptable limits, with the exception of the 20,000 ppm mixture in week 4. This only contained 40 - 44% of the nominal concentration, it was administered for 2 days only and the replacement diet was found to be acceptable. The average daily intakes in the two dietary groups were calculated to be as follows:

5000 ppm: M, 451 - 617 mg/kg bw/d

20,000 ppm: M, 2190 - 2310 mg/kg bw/d

Results are summarized in Table 6.8.2-1. There were no mortalities. In rats receiving 20,000 ppm there was an increase in hair loss from day 3 and unkempt coats from day 14. All groups receiving bupirimate had a lower body weight gain than the control group; this was apparent from week 1 onwards. Food consumption was only reduced in those groups receiving bupirimate via the diet. The amount of food scattered by rats receiving 20,000 ppm was higher than that scattered by controls rats, indicating unpalatable nature of this dietary formulation. Food conversion efficiency was decreased in the group receiving 20,000 ppm, and also in the last week of the 2 intermediate groups ($p<0.05$). The decreased body weight gain, food consumption and food conversion efficiency were particularly notable in the first week of treatment for the animals receiving 20,000 ppm bupirimate.

Thyroxin (T_4) levels were lower in rats receiving 5000 or 20,000 ppm bupirimate in the diet after 14 days; after 28 days this effect was also noted in the gavage group (equivalent to 5000 ppm dietary). Tri-iodothyronine (T_3) levels were not affected by treatment; therefore the ratio of T_4 to T_3 was lower in treated rats than in controls. There were no obvious effects of bupirimate on thyrotropin (TSH) levels, although there was much individual variation in the results. Uptake of I^{125} into the thyroid was higher in all treated groups compared to controls; the highest levels in treated animals were in particular seen after 6 hours, whereas uptake in controls was highest after 24 hours. After 28 days of treatment there was an increase in body weight-related thyroid weight in all treated groups but, most notably, in those receiving 20,000 ppm. A number of pathological changes were found in the thyroids of all treated groups. Administration of bupirimate was associated with a decrease in the proportion of follicles containing colloid, hypertrophy and slight hyperplasia of thyroid follicular epithelium, the development of follicular peninsulae, a decrease in follicular diameter, a decrease in the amount of colloid present in each thyroid cross section and a decrease in the intensity of eosinophilia of the colloid. All criteria were more markedly affected at 20,000 ppm and, in addition, this dose level was also associated with flattening or atrophy of the follicular epithelium of some follicles. The author concluded that the action of bupirimate on the thyroid was possibly related to a blockage of the incorporation of iodine into thyroxin, leading to a type of hypothyroid non-toxic goitre (i.e. thyroid enlargement, not resulting from inflammation or neoplasm, with a decreased hormone output). This hypothesis was supported by the clinical signs (sparse hair coat and decreased protein synthesis in the growing animal, seen as decreased weight gain and less efficient food conversion), decreased T_4 levels in blood plasma, increased iodine uptake by the thyroid and increased bodyweight-relative thyroid weight and the morphological alterations.

Table 33. Results of a 28-day oral toxicity study in male rats

Dose (ppm)	0	5000 / ~ 5000		20,000 (diet)	dr
		diet	gavage	diet	
Mortality	none				
Clinical signs					
ungroomed appearance				i	
losing hair (genitalia)				i	
food scattered				i	
Body weight gain (% of control-100)		-18***	-12***	-61***	dr
food intake (% of control-100)		-8	-2	-27***	dr
food conversion ratio (mean 4 wks)	3.0	3.3	3.3	5.6	dr
Thyroid function (28 days)					
TSH	3.9	6.5*	3.9	4.0	
T3	1.8	1.9	1.7	2.0	
T4	3.5	2.8*	2.9	2.3***	dr
T4/T3 ratio	1.9	1.5	1.7	1.2	dr
I^{125}-uptake (6h)	6.4	10.3*	11.4**	7.6	

Dose (ppm)	0	5000 / ~ 5000		20,000 (diet)	dr
		diet	gavage	diet	
I^{125}-uptake / g thyroid - 6h	242	470**	463**	446**	
- 48 h	323	422	475*	424	
Organ weights (% increase)					
thyroid	+20	+24	+23	+18	
Rel. thyroid	+6	+8*	+7	+9**	dr
Histopathology (mean value of all thyroids per group):					
% follicles with colloid	77	47	56	32	dr
Hypertrophy (score A)	1.3	2.5	2.6	3.4	dr
Mitoses per 10 HP fields	0.35	0.75	0.55	0.47	
Follicular peninsulae (score A)	1.2	2.3	3.0	3.6	dr
Follicular diameter (score B)	1.7	2.7	3.0	3.3	dr
Colloid amount (score B)	1.6	2.9	2.8	3.2	dr
Colloid pallor (score A)	1.1	2.7	3.0	3.3	dr

i = increased; dr = dose related, *p<0.5, **p<0.01, ***p<0.001
food conversion ratio = g food / g bodyweight gain
score A: 1= none; 2 = slight; 3=moderate; 4 = marked
score B: 1=normal; 2= slightly smaller; 3= moderately smaller; 4= markedly smaller

More details are available in the DAR.

Administration of bupirimate to the male rat, at 5000 ppm (equal to 450 mg/kg bw/d) or higher levels, whether administered by oral gavage or in the diet, was associated with changes in the thyroid. These were morphological changes indicative of hypothyroidism, a decrease in thyroxin (T_4) levels and a greater demand for I^{125} in the treated thyroid. The NOAEL for these effects on the thyroid could not be established, the LOAEL is 450 mg/kg bw/d.

4.10.1.4 Human information

No human information is available.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazards of bupirimate were assessed in the Draft Assessment Report and Proposed Decision of the Netherlands prepared in the context of the possible inclusion of bupirimate in Annex I of Council Directive 91/414/EEC (Draft Assessment Report, November 2009 concerning the placing of plant protection products on the market.

The summaries included in this proposal are copied from the DAR (and its addenda and assessment reports when these contain updated information). For an overview of the hazard property being evaluated, all reliable information relating to that property has been summarized in a table. Detailed information is only included for the key study used to derive the classification. References to individual studies are not included. For more details the reader is referred to the DAR and its addenda.

5.1 Degradation

Table 34: Summary of relevant information on degradation

Method	Results	Remarks	Reference

Bupirimate hydrolysis, SETAC guidelines	DT50 > 30 days	Hydrolytically stable	DAR (Ridge, 1999)
Bupirimate photodegradation, SETAC guidelines	DT_{50} 0.02 days		DAR (Lewis, 2000)
Bupirimate, OECD301 B	Not readily biodegradable	25.5% degradation after 28 days	DAR (Fiebig, 2008)
Bupirimate, water/sediment simulation study, SETAC guidelines	DT_{50} system 48.3 days		DAR (Haynes, 2003. Thomas, 2009a)

5.1.1 Stability

Hydrolysis

A hydrolysis study (Ridge, 1999) was conducted according to SETAC guidelines under GLP conditions. Pyrimidine-2-^{14}C-bupirimate was incubated in sterile buffers at a temperature of 20 ± 2 ºC at three pH levels (5,7 and 9) and at one concentrations (10 µg/L). Bupirimate is hydrolytically stable at pH 5, 7 and 9 at 22 ºC (DT50 value was > 30 days). Within 30 days, concentration of bupirimate showed a decline of 6-14%. The primary degradation product ethirimol was also shown to be hydrolytically stable.

Photodegradation in water

In a photodegradation study (Lewis, 2000), the DT_{50} value of bupirimate in artificial light was 0.02 summer sunlight days. Ethirimol and 1-ethylguanidine were identified as degradation products, with maximum levels of 56 and 6% of initial bupirimate. Two other products were formed at a level of > 10% of initial bupirimate, but were not further characterised.

Other studies, involving outdoor incubations under non-standardised conditions, are also indicative of fast photolysis of bupirimate. Apart from ethirimol and ethylguanidine, a number of other degradates was observed, including de-ethylated bupirimate and some bupirimate isomers. The latter may be further degraded to ethirimol. Ethirimol was shown to photolyse slowly under outdoor conditions. Main degradation products identified as ethylguanidine, urea and 4-n-butyl-pyradolidin-3,5-dione and additional minor compounds were 2-amino-4-valeroyl-4-methyl-oxazol-5-one and guanidine. A quantitative estimate of the photodegradation rate could not be obtained.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Biodegradation estimations are not provided since screening tests and simulation tests are available.

5.1.2.2 Screening tests

An OECD 301B study (modified Sturm test) is available for bupirimate. The CO_2 evolution in the control (57.5 mg/L after 29 days) satisfied the validity criterion for this study (<70 mg/L). The pass

level for the reference substance (60% degradation) was reached within 14 days. Bupirimate showed an average degradation of 25.5% after 28 days as CO_2 evolution. Based on the results bupirimate is considered not readily biodegradable.
In an OECD 301C study (MITI test) the ready biodegradability of ethirimol was assessed. The degradation of ethirimol after 28 days was 0% (as BOD), the BOD of the reference substance (sodium acetate) was 66% of its theoretical value. Based on these results ethirimol is considered not readily biodegradable.

5.1.2.3 Simulation tests

Water/sediment
One reliable water/sediment study with two types of aerobic water-sediment systems is available (Haynes, 2003; Thomas 2009a). One system was loam sediment (Bury pond, Cambridgeshire, UK) and the other system was sandy loam sediment (Emperor lkake, Chatsworth, UK).
Sediment samples were wet sieved (2 mm), water was 0.2 mm sieved. Incubation flasks (1 L) were filled with ca. 2.5 cm sediment (125 g dw, corresponding to 251.5 g ww for loam sediment and 219.1 g ww for sandy loam sediment), corresponding water was added to a total volume of 500 (sediment content 25%). Systems were equilibrated for 28 days at 20 ± 2 °C in the dark.
As stock solution of 0.084 mg/L bupirimate was prepared and 0.5 mL was added to the overlaying waterto give a final concentration of 0.0844 mg/L.
The systems were incubated at 20 ± 2 °C in the dark. Water and sediment of single flasks were sampled after 0, 1, 4, 7, 14, 29, 70 and 120 days. Volatiles were trapped in ethyl digol and 1 M NaOH (two traps).
Surface water was decanted from the flasks and analysed by LSC, HPLC-UV (254 nm) and TLC. Sediment was sequentially extracted with acetonitrile and acetonitrile/water (4:1, v/v). From day 14 onwards, an additional extraction involving refluxing the sediment with acetonitrile/water (9:1, v/v) for 6 hours was performed. Extracts were analysed by LSC, HPLC and TLC. Additional identification of metabolites on day 120 sediment extracts was performed by HPLC-MS after clean-up on a C_{18} column. Bound residues were determined by LSC after combustion. Non-extractable radioactivity in the 29, 70 and 120 day sediment samples was separated in humin, humic acid and fulvic acid fractions, Radioactivity in the trapping solutions was determined by LSC.

Dissipation of bupirimate from the water phase was influenced by sorption. The data suggest that degradation of bupirimate mainly occurs in the sediment phase and that the sorption to sediment is the limiting factor for the degradation rate. Bound residues increased to 61-66% after 120 days. Mineralisation was 0.3-3% after 70-120 days. The average DT_{50} of the whole system is 42.3 days. (see Table 29).

Table 35: DT50 values for [^{14}C-bupirimate in water/ sediment systems.

System	Water[a] DT_{50} [d]	Sediment[a] DT_{50} [d]	System[a] DT_{50} [d]
Bury pond	3.6[b]	41.4	37.1
Emperor lake	6.5	51.6	48.3
Geometric mean	4.8	46.2	42.3

[a] Disappearance due to degradation and mass transfer.
[b] Based on DFOP model (Double First-Order in Parallel).

Metabolite ethirimol, de-ethylated ethirimol and methoxy-hydroxy-methyl ethirimol were identified, but none of these reached levels of > 10% of bupirimate in the water and/or sediment

phase, and were not >5% of bupirimate on two consecutive time-points. The maximum level of ethirimol was found after 70 days and levels declined towards the end of the study.

5.1.3 Summary and discussion of degradation

Bupirimate is hydrolytically stable, a DT50 value of >30 days was calculated. Bupirimate is photolysed rapidly in an aqueous solution, the DT50 value in artificial light was 0.02 summer sunlight days at 54 °N.
Bupirimate was not readily biodegradable in a biodegradation screening test, neither was the major degradation product ethirimol. In a water/sediment degradation simulation test, the average DT50 of the whole system is 42.3 days. Mineralisation was less than 3% after 120 days. Based on these findings bupirimate is considered to be not rapidly degradable (CLP) and not readily degradable (DSD) in the aquatic environment.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The sorption of bupirimate was determined in a batch equilibrium experiment with five soils (Geffke, 2001). Soils were air dried and 2-mm sieved. Soil samples (1, 2 or 5 g) were shaken with 50 mL test solution (0.1, 0.5, 1.0, 5.0 and 10.0 mg/L). A summary of the results is given in Table 30.

Table 36: Summary of Freundlich adsorption parameters for bupirimate

Soil	K_F (L/kg)	1/n	R^2	OC (%)	Koc (L/kg)
Loamy sand	125	0.8705	0.9986	4.32	2822
Clay	87	0.9371	0.9980	3.29	2644
Loam	42	0.9395	0.9988	3.32	1265
Silt	12	1.0165	0.9963	1.36	882
Silt loam	43	0.9417	0.9986	2.39	1799

The sorption of ethirimol was determined in a batch equilibrium experiment with four soils (Dyson et al., 1992). Soils were air dried and 2-mm sieved. Soil samples (4 g) were shaken with 50 mL test solution (0.04, 0.1, 0.2, 1.0 and 5.0 mg/L). A summary of the results is given in Table 31.

Table 37: Summary of Freundlich adsorption parameters for bupirimate

Soil	K_F (L/kg)	1/n	OM (%)	Koc[1] (L/kg)
Clay loam	33	0.8736	6.0	950
Sandy loam	2.3	0.8078	2.3	170
Sandy loam	3.0	0.8828	5.3	97
Sand	1.8	0.8312	0.8	390

[1] Calculated as: Kom × 1.724

5.2.2 Volatilisation

Bupirimate has vapour pressure of 1.31 x 10^{-4} Pa (extrapolated value at 25ºC), and a Henry's law constant of 1.35 $Pa.m^3mol^{-1}$. Based on this information it is considered that volatilisation of bupirimate may occur but concentrations will be generally low.

5.2.3 Distribution modelling

Not relevant for this type of dossier.

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

Table 38: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 305, GLP	BCF 173-185 L/kg (not lipid normalized) BCF 80-128 L/kg (lipid normalized)	Concentration 1.0 and 10.0 µg/L nominal	DAR (Swales, 2002)

5.3.1.1 Bioaccumulation estimation

Bupirimate has a log Kow value of 3.68 ± 0.22 at 21 ± 1°C and pH 6.92

5.3.1.2 Measure bioaccumulation data

In a study carried out according to OECD 305 guidelines under GLP conditions (Swales, 2002), the bioconcentration factor (BCF) of fish (rainbow trout) exposed to radiolabelled bupirimate were determined during 28 days of continuous exposure to 1.0 and 10.0 µg/L nominal concentration. Thereafter, the remaining fish were transferred to dilution water for a depuration period of 14 days. A control treatment consisted of a test vessel treated only with dilution water containing the solvent methanol. Bioconcentration factor was calculated by dividing fish residue concentration by mean measured concentration in test medium. Mean measured concentrations in the exposure water were 1.048 µg [14C]-bupirimate/L in the 1.0 µg/L treatment with a range of 0.713 to 1.445 µg/L and 10.18 µg/L in the 10.0 µg/L treatment with a range of 4.160 to 14.44 µg/L. During the exposure phase, the majority of the radioactivity was shown to be parent bupirimate. HPLC-analyses of water samples showed that unknown compounds covered 0 to 3% at the start of the exposure period and increased to 23 – 35% during the first week. Percentage unknown compounds stayed stable during the remaining exposure period. Total recovery of radioactivity in water samples ranged between 94 and 100 %. Lipid content of whole fish tissue ranged from 7.2 to 10.7 % wet weight, with the exception of two samples where the lipid content was 6.1 and 6.3 %. Uptake and depuration of radioactivity in fish tissue was rapid and similar in both test solutions. The time to eliminate 50% and 95% of ^{14}C from whole fish was 0.5 and 2 days, respectively, at both concentrations. Water quality criteria were met. Steady state concentrations in fish were reached within the experimental period. The bioconcentration factors are summarised in Table 33.

Table 39 Bioconcentration factors bupirimate

Concentration [µg/L]	BCF based on	whole fish (not lipid normalized)
1.0	steady state	181
	kinetics	173
10.0	steady state	179
	kinetics	185

5.3.2 Summary and discussion of aquatic bioaccumulation

Bupirimate has log Kow of 3.68. The highest whole fish BCF derived for bupirimate was 185 L/kg (not lipid normalized). The lipid content of whole fish ranged from 7.2-10.7% except in two fish which had 6.1 and 6.3% lipid content. Taken the lower lipid value into account, it is assumed that after lipid normalisation to 5% the BCF value of bupirimate remains above 100. Bupirimate is therefore assumed to meet the criterion for bioaccumulation potential according to Directive 67/548/EEC since it is above 100. In contrast, the BCF value does not fulfil the criterion for Regulation EC1272/2008, since it does not exceed the value of 500.

5.4 Aquatic toxicity

A brief summary of the aquatic toxicity studies listed in the DAR for the three trophic levels fish, aquatic invertebrates and algae/aquatic plants are reported below. Only reliable and acceptable ecotoxicity tests from the Draft Assessment Report were used.

Table 40: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference

Acute fish No guideline, non GLP	LC50 1.25-2.5 mg/L	96-h, flow-through, nominal, *Oncorhynchus mykiss*	DAR (Hill et al., 1975a)
Acute fish No guideline, non GLP	LC50 1.0-1.5 mg/L	96-h, flow-through, nominal *Oncorhynchus mykiss*	DAR (Hill et al., 1975b)
Acute fish No guideline, non GLP	LC50 1.25-2.5 mg/L	96-h, flow-through, nominal, *Lepomis macrochirus*	DAR (Hill et al., 1975a)
Acute fish No guideline, non GLP	LC50 1.0-1.5 mg/L	96-h, flow-through, nominal, *Lepomis macrochirus*	DAR (Hill et al., 1975b)
Chronic fish OECD 204 guideline, GLP	NOEC 0.30 mg/L	28-d, flow-through, measured, *Oncorhynchus mykiss*	DAR (Sankey et al., 1990)
Chronic fish OECD 210 guideline, GLP	NOEC 0.10 mg/L	32-d, semi-static, nominal, *Pimephales promelas*	DAR (Scheerbaum, 2007)
Acute invertebrates, OECD 202 guideline, GLP	EC50 >3.41 mg/L	48-h,static, measured, *Daphnia magna*	DAR (Mattock, 2002)
Chronic invertebrates, OECD 202 guideline, GLP	NOEC 0.56 mg/L	21-d, semi-static, measured, *Daphnia magna*	DAR (Stewart et al., 1991)
Algae inhibition, OECD 201 and U.S. EPA EG8 guidelines, GLP	ErC50 2.5 mg/L NOErC 0.32 mg/L	96-h, static, nominal, *Pseudokirchneriella subcapitata*	DAR (Hanstveit, 1989)

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Toxicity of bupirimate to rainbow trout (*Oncorhynchus mykiss*) and bluegill sunfish (*Lepomis macrochirus*) was tested under flow-through conditions (Hill, 1975b). The fish were commercially obtained and acclimated to test conditions 11.5 days. Test substance was dissolved in acetone (5 g/L). Nominal concentrations tested ranged from 1.0 to 68 mg/L in rainbow trout and 1.0 to 15 mg/L in bluegill sunfish. Solvent control (acetone, 5 g/L) was included. Mean measured concentrations at 1.0 to 15 mg/L ranged from 79 to 107 % of nominal. In rainbow trout, no mortalities were observed in the control and the 1.0 mg/L treatments over 96 hours. The time to 100% mortality at 1.5 mg/L was 57.8 hours. In bluegill sunfish, no mortalities were observed in the control and the 1.0 mg/L treatments over 96 hours. Time to 100% mortality at 1.5 mg/L was 61.8 hours. For both species, the nominal 96-h LC_{50} is between 1.0 and 1.5 mg/L.

In a second study also conducted by Hill et al. (1975a), nominal 96-h LC_{50} values between 1.25 and 2.5 mg/L were obtained in rainbow trout and bluegill sunfish. Nominal concentrations tested ranged from 1.25 to 20 mg/L in both species. Mean measured concentrations at 1.25 to 10 mg/L ranged from 90 to 108% of nominal.

5.4.1.2 Long-term toxicity to fish

Toxicity of bupirimate to rainbow trout *Oncorhynchus mykiss* was determined in a flow-through system for 28 days in a GLP-compliant OECD 204 guideline test (Sankey, 1990). Nominal concentrations were 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L. Ten fish per concentration and in the solvent (DMF) control were used, in the dilution water control 9 fish were used. Mean measured concentrations ranged from 93 to 111 % of nominal in the test vessels. NOEC values for mortality could not be statistically determined due to lack of replications. However, since mortality showed very consistent trends with concentration and time, NOEC values are estimated to be 0.49 mg/L after 14 days and 0.30 mg/l after 28 days exposure on basis of mean actual concentrations. NOEC values on basis of length or weight after the 28 days exposure period are estimated to be 0.56 mg/L for both length and weight.

.A 32-day fish early life stage flow-through study (Scheerbaum, 2007) was undertaken with fathead minnow (*Pimephales promelas*). Newly fertilised eggs (1 hour post fertilisation, four replicates/concentration, 15 eggs/replicate) were exposed to Bupirimate (94.1% pure) at nominal concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8 mg/L plus control and solvent control (DMF). The total duration of the exposure period was 32 days (28 days post-hatch). Mean measured concentrations represented 98-105% of nominal concentrations. Total egg fertilisation of the spawn was >95%. Percent hatch was not significantly affected in any of the test concentrations when compared to the pooled control group. Post hatch survival at the end of the study was not statistically significantly affected at any concentration. Due to a technical malfunction in the dosing system for the highest test concentration, high mortality occurred on post-hatch day 16; all surviving larvae were transferred to one replicate on post-hatch day 17. This group was excluded from further evaluations. At the end of the test, overall survival was statistically significantly reduced at 0.2 and 0.4 mg/L. Mean fish length and fish dry weight were statistically significantly reduced at 0.4 mg/L. Biologically significant morphological and behavioural effects were not observed in any of the test concentrations. The NOEC was 0.10 mg/L, based on a significant effect on overall survival at and above 0.2 mg/L (based on nominal concentrations).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

The acute toxicity to *Daphnia magna* was tested in a GLP-compliant OECD 202 guideline test under static conditions (Mattock, 2002). Five daphnia (<24 h old) were used per vessel in quadruplicate. Nominal concentrations of the test substance ranged from 0.5 to 16 mg/l. Determination of actual concentrations was carried out at start en termination of the study. Mean measured concentrations ranged from 118% at the lowest concentration to 21% at the highest concentration. The decline in the measured concentrations in the higher treatments was considered to be due to limited solubility of bupirimate (18 mg/l at pH 7.3, 3.41 mg/l in ASTM medium). No undissolved material was observed in the test wessels. After 24 hours, one immobile daphnia was observed in the solvent control and at 2.0 mg/l. No other immobilized daphnia were observed. The 48-h EC50 value is > 3.41 mg /L (measured concentrations).

5.4.2.2 Long-term toxicity to aquatic invertebrates

Chronic toxicity of bupirimate to < 24 h old *Daphnia* was determined in a semi-static system for 21-d (Stewart, 1991). Mortality was recorded on daily basis, offspring was scored at renewal of the solutions. At test termination, length of parent daphnia was measured.
Control, solvent control, 0.1, 0.18, 0.32, 0.56, 1.0 and 1.8 mg/L. Solvent control and treatments contained 0.09 mL triethylene glycol/L. 10 replicates for controls and treatments, each replicate contained one daphnid.
No significant differences between concentrations in fresh and old solutions were observed. Mean actual concentrations were 0.10, 0.18, 0.30, 0.56, 0.94 and 1.8 mg/L (94 – 100 % of nominal). No mortality was observed in any of the treatments during the whole test period. Number of offspring was significant lower in the 1.0 and 1.8 mg/L treatments. Validity criteria were met. NOEC was determined to be 0.56 mg/L based on mean measure concentrations on basis of reproduction.

5.4.3 Algae and aquatic plants

The acute toxicity to *Pseudokirchneriella subcapitata* was tested in a GLP-compliant OECD 201 guideline test under static conditions (Hanstveit, 1989). Eight concentrations ranging from 0.01 to

3.2 mg/l (nominal), a control and solvent control (dimethylsulphoxide) were tested. Concentrations of the test substance were analysed in replicate flasks without algae. Actual concentrations were 106% and 74% at begin and end of the study, respectively. As chemical analysis was carried out in the absence of algae, the measured concentration may have underestimated the actual concentrations. The control algae were considered not to be in log-phase for the whole testing period. The 96-h ErC50 and NOErC value are 2.50 and 0.32 mg /L based on mean measured concentrations, respectively.

5.4.4 Other aquatic organisms (including sediment)

No data available.

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

CLP- Acute aquatic hazards

The lowest available L(E)C_{50} value for bupirimate is 1.0-1.5 mg/L obtained in fish. In this study, no mortality was observed at 1.0 mg/L during the study period whereas all the fish died at 1.5 mg/L, suggesting that the LC_{50} value is greater than 1 mg/L with a steep dose-response curve. In a second study carried in fish, 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.

CLP - Aquatic chronic hazards

Bupirimate is considered not rapidly degradable. Bupirimate does not fulfil the criterion of BCF > 500. The lowest NOEC of 0.10 mg/L was obtained in fish. The NOEC value of 0.10 mg/L falls within the range 0.01 < NOEC ≤ 0.1 mg/L. Being not rapidly degradable, bupirimate therefore fulfils criteria for classification as Aquatic Chronic Cat. 1 with an M-factor of 1.

Directive 67/548/EEC

Bupirimate is not readily degradable and has a BCF value above 100 L/kg. The lowest available L(E)C_{50} value for bupirimate is 1.0-1.5 mg/L obtained in fish. In this study, no mortality was observed at 1.0 mg/L for 96-hours whereas all the fish died ad 1.5 mg/L, suggesting an LC_{50} value which is greater than 1 mg/L with a steep dose-response curve. Being not readily degradable and based on an LC_{50} value between 1 and 10 mg/L, bupirimate fulfils the criteria for classification with N; R51/53.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Table 41 Conclusion on environmental classification

	CLP Regulation	**Directive 67/548/EEC (DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Aquatic Chronic 1 (H410) Chronic M-factor 1	N; R51-53

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.

Supplemental information - In depth analyses by RAC

Summary of key studies' results reported in the CLH report:

Method	Results	Remarks
Bioaccumulation OECD 305	Lipid norm. BCF 80–128 L/kg	Concentration 1.0 and 10.0 µg/L nominal
Hydrolysis SETAC guideline	DT50 > 30 days	Hydrolytically stable
Photodegradation SETAC guideline	DT_{50} = 0.02 days	Photodegradable
Biodegradation OECD301 B	Not readily biodegradable	25.5% degradation after 28 days
Biodegradation water/sediment simulation SETAC guidelines	DT_{50} system = 48.3 days	Disappearance due to degradation and mass transfer. Mineralisation was less than 3% after 120 days.
Acute fish No guideline, non GLP	LC50 = 1.0–1.5 mg/L	96-h, flow-through, nominal *Oncorhynchus mykiss* and *Lepomis macrochirus*
Chronic fish OECD 210, GLP	NOEC = 0.10 mg/L	32-d, semi-static, nominal *Pimephales promelas*
Algae inhibition OECD 201 and EPA EG8, GLP	ErC50 = 2.5 mg/L NOErC = 0.32 mg/L	96-h, static, nominal *Pseudokirchneriella subcapitata*

6 OTHER INFORMATION

This proposal for harmonised classification and labelling is based on the data provided for the registration of bupirimate according to Directive 91/414/EEC. The summaries included in this proposal are partly copied from the DAR volume 3, annex B. Some details of the summaries were not included when considered not relevant for a decision on the classification and labelling of this substance. For more details the reader is referred to the DAR Volume 3 and its addendum.

7 REFERENCES

European Commission. Draft Assessment Report Bupirimate, prepared by The Netherlands April 2007.

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8 ANNEXES