Committee for Risk Assessment

RAC

Annex 1

Background document

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

iprovalicarb (ISO); isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate

EC Number: -
CAS Number: 140923-17-7

CLH-O-0000001412-86-237/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

30 November 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate (ISO name: Iprovalicarb)

EC Number:

CAS Number: **140923-17-7**

Index Number:

Contact details for dossier submitter: PRCD DAFM Ireland

Version number: 3 **Date:** **02/10/2017**

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON IPROVALICARB (ISO); ISOPROPYL [(2S)-3-METHYL-1-{[1-(4-METHYLPHENYL)ETHYL]AMINO}-1-OXOBUTAN-2-YL]CARBAMATE

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate (ISO name: Iprovalicarb)
EC number:	Not allocated
CAS number:	140923-17-7
Annex VI Index number:	Not allocated
Degree of purity:	95.00%
Impurities:	Confidential Information. None considered to have a relevant impact on classification.

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation
Current entry in Annex VI, CLP Regulation	No current entry, not classified.
Current proposal for consideration by RAC	Carc. 2; H351
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Carc. 2; H351

1.3 Proposed harmonised classification and labelling based on CLP Regulation.

Table 3: Proposed classification according to the CLP Regulation

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON IPROVALICARB (ISO); ISOPROPYL [(2S)-3-METHYL-1-{[1-(4-METHYLPHENYL)ETHYL]AMINO}-1-OXOBUTAN-2-YL]CARBAMATE

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	None.		-	Conclusive, but not sufficient for classification
2.2.	Flammable gases	None.		-	Data lacking
2.3.	Flammable aerosols	None.		-	Data lacking
2.4.	Oxidising gases	None.		-	Data lacking
2.5.	Gases under pressure	None.		-	Data lacking
2.6.	Flammable liquids	None.		-	Data lacking
2.7.	Flammable solids	None.		-	Conclusive, but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None.		-	Data lacking.
2.9.	Pyrophoric liquids	None.		-	Data lacking
2.10.	Pyrophoric solids	None.		-	Data lacking
2.11.	Self-heating substances and mixtures	None.		-	Conclusive, but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None.		-	Data lacking
2.13.	Oxidising liquids	None.		-	Data lacking
2.14.	Oxidising solids	None.		-	Conclusive, but not sufficient for classification
2.15.	Organic peroxides	None.		-	Data lacking
2.16.	Substance and mixtures corrosive to metals	None.		-	Conclusive, but not sufficient for classification
3.1.	Acute toxicity - oral	None.		-	Conclusive, but not sufficient for classification
	Acute toxicity - dermal	None.		-	Conclusive, but not sufficient for classification
	Acute toxicity - inhalation	None.		-	Conclusive, but not sufficient for classification
3.2.	Skin corrosion / irritation	None.		-	Conclusive, but not sufficient for classification
3.3.	Serious eye damage / eye irritation	None.		-	Conclusive, but not sufficient for classification
3.4.	Respiratory sensitisation	None.		-	Data lacking

3.4.	Skin sensitisation	None.		-	Conclusive, but not sufficient for classification
3.5.	Germ cell mutagenicity	None.		-	Conclusive, but not sufficient for classification
3.6.	Carcinogenicity	CARC. 2; H351		-	
3.7.	Reproductive toxicity	None.		-	Conclusive, but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None.		-	Conclusive, but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	None.		-	Conclusive, but not sufficient for classification
3.10.	Aspiration hazard	None.		-	Data lacking
4.1.	Hazardous to the aquatic environment	None.			Conclusive, but not sufficient for classification
5.1.	Hazardous to the ozone layer	None.			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:

Pictogram: GHS08

Signal word: Danger
Hazard statements: H351
Precautionary statements: Not proposed (not included in Annex VI Reg EC 1272/2008)

Proposed notes assigned to an entry:

None.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Iprovalicarb is an active substance in the meaning of EU Directive1107/09and therefore is subject to harmonised classification and labelling (Regulation EC no. 1272/2008). Iprovalicarb has not been previously considered for inclusion in Annex VI of Regulation (EC) 1272/2008.

Evaluations carried out under other regulatory contexts

US EPA: Cancer Assessment Review Committee (CARC) (Feb 2002);

The CARC of the Health Effects Division (HED) of the Office of Pesticides Programs evaluated the carcinogenic potential of iprovalicarb. This meeting was also attended by the Canadian Pest Management Regulatory Agency (PMRA). The studies assessed in this HED report were the same as those assessed in this (AIR II) evaluation with the exception of the PCNA study, which was apparently later reviewed by the Californian EPA (January, 2012).

The CARC classified iprovalicarb according to the EPA draft Guidelines for Carcinogenic Risk Assessment (July 1999) in to the category 'Likely to be carcinogenic to humans' based on the following weight-of-evidence considerations:

- Iprovalicarb induced rare and infrequently occurring tumours in rats. At the high dose, males developed osteosarcomas and females developed transitional cell papillomas of the urinary bladder. At the mid and high doses, females developed mixed Mullerian tumours of the uterus and follicular cell adenomas/carcinomas of the thyroid gland. Although incidences of these tumours were low, they are either rare or uncommon in this strain of rat. Most of these tumours were induced above the limit dose which was not excessively toxic. In mice, no treatment-related increase in tumours was observed in animals treated above the limit dose which was adequate and not excessively toxic.

- Iprovalicarb is not mutagenic. Although mechanistic studies suggested that iprovalicarb is not a tumour initiator, these studies did not establish the mode of action for tumour induction in rats

2.2 Short summary of the scientific justification for the CLH proposal

Human Health CLH proposal:
Rare tumours were increased in four different organs in a possibly treatment-and dose-related manner in the rat. Carc. 2 is recommended on the basis of limited evidence for a carcinogenic potential from a single species exposed long-term to a significantly high dose.

Environmental toxicology CLH proposal:
Iprovalicarb does not classify under CLH based on the following aquatic endpoints:

Acute toxicity fish > 20.7 mg/L (mm)
Chronic toxicity fish ≥ 9.89 mg/L (nom)
Bioaccumulation fish = 10
Log kow = 3.18 (Diastereomer A)
Log kow = 3.2(Diastereomer B)
Acute toxicity invertebrate > 19.8 mg/L (mm)

Chronic toxicity invertebrate 1.89 mg/L (mm)
Chronic toxicity sediment dwelling organism $EC_{15emerg}$ 128 (nom)
Acute toxicity algae > 10 mg/L (nom)
Acute toxicity aquatic-plants Not required

2.3 Current harmonised classification and labelling

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

No current Annex VI entry.

2.4 Current self-classification and labelling

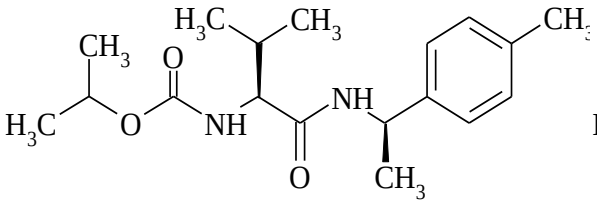
2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

No classification.

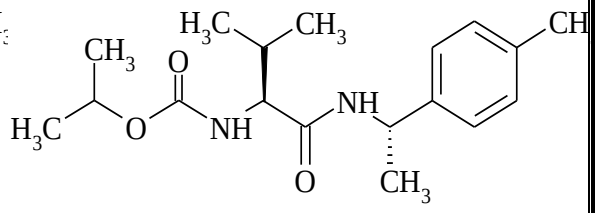
RAC general comment

Iprovalicarb is an active substance in the meaning of EU Directive 1107/09 (the substance is used as a foliar-applied fungicide) and therefore is subject to harmonised classification and labelling (Regulation EC no. 1272/2008). Iprovalicarb has not been previously considered for inclusion in Annex VI of Regulation (EC) 1272/2008. The substance is defined as the sum of two diastereoisomers; their absolute configuration is given below:

Diastereoisomer A (*SR*)



Diastereoisomer B (*SS*)



3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Iprovalicarb is an active substance in the meaning of Directive 1107/09 and is therefore subject to harmonised classification and labelling (Regulation EC no. 1272/2008).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

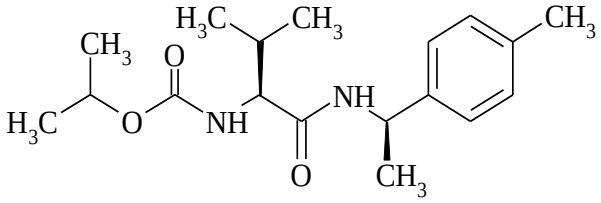
Table 5: Substance identity

EC number:	Not applied
EC name:	Not applied
CAS number (EC inventory):	Not applied
CAS number:	140923-17-7
CAS name:	Carbamic acid, [(1*S*)-2-methyl-1-[[[1-(4-methylphenyl)ethyl]amino]-carbonyl]propyl]-, 1-methylethyl ester
IUPAC name:	isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate
CLP Annex VI Index number:	none
Molecular formula:	$C_{18}H_{28}N_2O_3$
Molecular weight range:	320.4 g/mol

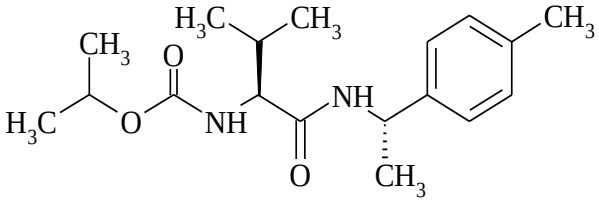
Structural formula:

Iprovalicarb is defined as the sum of two diastereoisomers; their absolute configuration is given below:

Diastereoisomer A (*SR*)



Diastereoisomer B (*SS*)



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Iprovalicarb	950 g/kg (mixture of two diastereoisomers)	min. 470 g/kg diastereoisomer A (SZX 0932): *SR*-enantiomer min. 480 g/kg diastereoisomer B (SZX 1097): *SS*-enantiomer	None.

Current Annex VI entry: None.

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
-	-	-	-

Current Annex VI entry: None.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	-	-	-	-

Current Annex VI entry: None.

1.2.1 Composition of test material

1.3 Physico-chemical properties

Technical Iprovalicarb consists of a 50:50 mixture of the SR and SS isomers. Iprovalicarb is a white odourless powder (solid) and the isomeric mixture has a minimum purity of 950 g/kg (95% w/w). The melting point of the mixed isomer Iprovalicarb is 163- 165°C [SR isomer 183°C, SS isomer 199°C] and its vapour pressure indicates that it is not volatile. Iprovalicarb is poorly soluble in water [17.8 mg/L] and is a moderately soluble in non-polar organic solvents [circa 0.1 g/l in n-hexane, 5.3 g/l in toluene]. The octanol/water partition co-efficient at log 3.2 indicates that Iprovalicarb is fat soluble and may have a tendency to bio-accumulate. Iprovalicarb is stable to hydrolysis in the pH range 5-9 and as it does not absorb light in the visible spectrum it does not degrade photolytically. Iprovalicarb is not flammable, oxidising or explosive and does not classify from a physical/chemical point of view.

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 material (both diastereomers): pure white powder (99.9 %) Technical material: white to yellow powder	Reference 1.	Observation.
Melting point	183°C (diastereomer *SR*) (97.5%) 199°C (diasteresomer *SS*) (99.4%) 163-165°C (mixture) (99.9%)	Reference 1.	Measured.
Boiling point	Not measurable, decomposition > 160°C (purity not stated).	Reference 1.	
Relative density	Density: 1.11 g/cm³ at 20°C	Reference 1.	Measured.
Vapour pressure	7.9×10^{-8} Pa at 20°C (isomer mix.) (99.9 %) - extrapolated 2.1×10^{-7} Pa at 25°C (isomer mix.) (99.9 %) - extrapolated	Reference 1.	Measured.
Surface tension	66 mN/ m at 20°C (saturated aq. solution) (Isomer mix) (99.9 %).	Reference 1.	Measured
Water solubility	11 mg/L at 20°C (Diastereomer SR) (97.5 %) 6.8 mg/L at 20°C (Diastereomer SS) (99.4 %) 17.8 mg/L at 20°C (isomer mix.) (99.9 %)	Reference 1.	Measured. There was no effect of pH. The solubility was not influenced by the pH in the range of pH 5.6 to pH 9.2.
Partition coefficient n-octanol/water	log $P_{O/W}$ = 3.18 at 20°C (diastereomer SR) (97.5%) log $P_{O/W}$ = 3.20 at 20°C (diastereomer SS) (99.4%) log $P_{O/W}$ = 3.2 at 20°C (isomer mix.) (99.9%)		Measured.

<table>
<tr><td>Flash point</td><td>Not applicable. The active substance is a solid; its melting point is > 40°C.</td><td>Reference 2.</td><td></td></tr>
<tr><td>Flammability</td><td>Not highly flammable (98.7 %)
Not auto-flammable (98.7 %)</td><td>Reference 2.</td><td>Measured.</td></tr>
<tr><td>Explosive properties</td><td>Not explosive (98.7 %)</td><td>Reference 2.</td><td>Measured.</td></tr>
<tr><td>Self-ignition temperature</td><td>Does not undergo spontaneous combustion in the sense of EU guideline A.16 and in the 1l Bowes-Cameron-Cage-Test. Iprovalicarb is not auto flammable.</td><td>Reference 2.</td><td>Measured.</td></tr>
<tr><td>Oxidising properties</td><td>Not oxidising (97.5 %)</td><td>Reference 3.</td><td>Measured.</td></tr>
<tr><td>Granulometry</td><td>Not investigated.</td><td></td><td></td></tr>
<tr><td>Stability in organic solvents and identity of relevant degradation products</td><td><u>Solubility at 20 °C in g/L (98.4 %):</u>
<table>
<tr><th>Solvent</th><th>SR</th><th>SS</th><th>Iprovalicarb</th></tr>
<tr><td>n-hexane</td><td>0.06</td><td>0.04</td><td>0.10</td></tr>
<tr><td>Toluene</td><td>2.9</td><td>2.4</td><td>5.3</td></tr>
<tr><td>Dichloromethane</td><td>97</td><td>35</td><td>132</td></tr>
<tr><td>2-propanol</td><td>15</td><td>13</td><td>28</td></tr>
<tr><td>1-octanol</td><td>11</td><td>9.3</td><td>20.3</td></tr>
<tr><td>Polyethylene glycol</td><td>2.0</td><td>1.8</td><td>3.8</td></tr>
<tr><td>Acetone</td><td>22</td><td>19</td><td>41</td></tr>
<tr><td>Dimethylformamide</td><td>63</td><td>55</td><td>118</td></tr>
<tr><td>Acetonitrile</td><td>8.1</td><td>11.0</td><td>19.1</td></tr>
<tr><td>Ethylacetate</td><td>13</td><td>7</td><td>20</td></tr>
<tr><td>Dimethylsulfoxide</td><td>27</td><td>15</td><td>42</td></tr>
</table></td><td>Reference 1.</td><td>Measured.</td></tr>
<tr><td>Dissociation constant</td><td>No dissociation.
(Iprovalicarb has no acidic or basic properties in aqueous solutions. It is not possible to specify dissociation constants for water).</td><td>Reference 1.</td><td></td></tr>
<tr><td>Viscosity</td><td>Not applicable.</td><td></td><td>Substance is a solid.</td></tr>
</table>

Reference 1: *Krohn, J. (12th January 1995 & revised 2nd September 1996): "Physical and chemical properties of SZX 0722". Bayer study 14 120 0831.*

Reference 2: *Mix, K.H. (13th March 1995). "Determination of safety-relevant parameters of SZX 0722 (Mischpartie: 05013/0212)". Report no.: 95/00049; PC 767.*

Reference 3: *Smeykal, H. (10th October 2008) "Iprovalicarb, technical substance: oxidising properties". Report no. 20080616.01. M-309852-01-1.*

2 MANUFACTURE AND USES

2.1 Manufacture

Confidential information.

2.2 Identified uses

The product (Iprovalicarb + Folpet WG 65.3) is used as a foliar-applied fungicide. Application will be by standard spray equipment according to good agricultural practice. For further details on the application of the product please refer to the national labels.

Best results are usually achieved when applied as a protectant fungicide or in the early stages of the pathogens' development. Therefore, the product should be applied at occurrence of first symptoms i.e., disease onset.

No waiting period applies for grapes as this is a permanent crop.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Based on the results obtained, classification and labelling for physical-chemical properties according to Regulation 1272/2008/EC (CLP). Iprovalicarb is not flammable, oxidising or explosive and does not classify from a physical/chemical point of view.

3.1 Conclusions on classification and labelling

None

RAC evaluation of physical hazards
Summary of the Dossier Submitter's proposal According to the Dossier Submitter (DS) Iprovalicarb is not flammable, oxidising or explosive and does not classify for physical hazards. **Comments received during public consultation** No comments were received during public consultation. **Assessment and comparison with the classification criteria** The DS's proposal is supported in two internal reports (Mix, 1995[1] and Smeykal, 2008[2]) that are not based on tests conducted under the usual guidelines. Nevertheless, in the absence of other relevant information, **RAC supports the DS's proposal for no classification of iprovalicarb regarding physical hazards.**

[1] Mix, 1995. "Determination of safety-relevant parameters of SZX 0722 (Mischpartie: 05013/0212)". Report no.: 95/00049; PC 767.

[2] Smeykal, 2008. "Iprovalicarb, technical substance: oxidising properties". Report no. 20080616.01. M-309852-01-1.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Iprovalicarb was readily absorbed and speedily eliminated from the body. More than 91% of the administered dose was absorbed from the GIT within 48 to 72 h after oral administration. The major route of elimination was faecal for male rats and about equally faecal and renal for females.

Biotransformation to volatile metabolites including CO_2 was negligible, being measured at 0.01% of the administered dose.

The main metabolic pathway was oxidation of the methyl group located on the aromatic ring leading to the final carboxylic acid metabolite *via* the hydroxymethyl-derivative. Some minor metabolites originated from cleavage of the molecule.

Metabolism was extensive in the rat. Less than 10% passed through as unchanged parent material in the low dose experiments and between 16 and 21% in the high dose. Twelve metabolites were identified, the main one being the diastereomer pair of iprovalicarb-carboxylic acid (M03). This pair alone accounted for 58% of the administered dose in all tests. Small amounts of eight other metabolites were detected in the urine. All of these together accounted for less than 2% each and additively to 7% at most of the administered dose. The radioactive components identified in the rat bile were M03 and two of its conjugates.

80% to 90% of the total radioactivity administered was identified.

In the supplementary study, the effect of dose and subchronic feeding was observed in rats. Rats were fed with 500 and 20,000 ppm in the diet for 2 days and 13 weeks. Subsequently, radioactive labelled compound was administered once. Absorption and excretion data from this study did not differ qualitatively to any great extent from those reported in the main study. ≈ 99% of the dose was excreted *via* urine and faeces within 72 h of dosing. Again, the major route of excretion was faecal for male rats and about equally faecal and renal for females. The concentration maximum in plasma was reached approximately 1 h after dosing. The maximum equivalent concentration in plasma was slightly reduced after sub-chronic feeding. High dose females had lower concentrations of total radioactivity in plasma than males.

No major effects on the general metabolic pattern of iprovalicarb were evident in this study when compared with the main study. The primary compound detected in all excreta was iprovalicarb carboxylic acid (M03). The SS:SR isomer ratio was shifted in favour of the SR isomer in the urine of rats which received 20,000 ppm a.s. in the feed compared to those receiving the lower dose of 500 ppm. The effect was much more noticeable in males than in females; a very minimal effect was noticed in the faeces.

The higher dose groups also exhibited higher proportions of unchanged parent compound in the faeces. Also, higher amounts of parent were detected in the faeces of females after subchronic feeding. There was no shift in the isomer ratio of the excreted unchanged parent compound.

4.1.2 Human information

None available.

4.1.3 Summary and discussion on toxicokinetics

See above

4.2 Acute toxicity

Table 11: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Oral LD_{50} Rat male/female Vehicle: water/ cremophor 2%v/ Iprovalicarb: 98.4%	> 5000 mg/kg bw	Guideline study No clinical signs	Bomann, 1993; 22110 RAR B.6.2.1.1
Oral LD_{50} Mouse male/female Vehicle: water/ cremophor 2%v/v 5000 Iprovalicarb: 98.4%	> 5000 mg/kg bw	Guideline study No clinical signs	Bomann, 1993; 22108 RAR B.6.2.1.2
Acute dermal rat (Bor:WISW) Vehicle: 2% Cremophor EL in physiological saline Iprovalicarb: 98.4% pure	LD_{50} > 5000 mg/kg bw	Guideline study No clinical signs	Bomann, W. 1993 Study No. T4041135 RAR B.6.2.2
Acute inhalation rat (Bor:WISW) Iprovalicarb: 97.6%	LC_{50} > 4977 mg/m^3 air		Pauluhn, J. 1993 Study No. T9071167 RAR B.6.2.3

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Rat:

Iprovalicarb (98.4% purity) was administered *p.o.* by means of an oesophageal tube, formulated in 2% Cremophor EL V/V in demineralised water, to 5/sex/group fasting rats (Bor: WISW [SPF Cpb]). Due to the limitations of the highest producible concentration of iprovalicarb the administration of the single 5000 mg/kg bw dose was achieved by two separate deliveries of 20 ml/kg bw each, at approximately 6 hour intervals.

No mortalities occurred during the course of this study. All animals (5/sex) showed signs i.e. light coloured faeces. This was as a result of the administration of the white test material. This was noted from 6h - 2d following treatment. There were no indications of treatment-related systemic toxicity. There were no effects on animal body weights.

The acute oral LD_{50} (rat) > 5000 mg/kg bw for both sexes.

Mouse:

Iprovalicarb (98.4% purity) was administered *p.o.* by means of an oesophageal tube, formulated in 2% Cremophor EL V/V in demineralised water, to 5/sex/group fasting mice (Bor: NMRI [SPF-Han]). Due to the limitations of the highest producible concentration of iprovalicarb, the administration of the single 5000 mg/kg bw dose was achieved by two separate deliveries of 20 ml/kg bw each, at approx. 6 hour intervals.

No mortalities occurred during the course of this study and no animals showed any signs of treatment-related effects. No significant effects on body weight were noted and there were no test substance-related gross pathological changes observed at necropsy.

The acute oral LD_{50} (mouse) > 5000 mg/kg bw

4.2.1.2 Acute toxicity: inhalation

Five male and five female Wistar rats (Bor: WISW [SPF-Cpb]) were exposed, head/nose only, to a dust of the test substance (Iprovalicarb 97.6% purity) at 0, 505, and 4977 mg/m^3 air for 4 hours. Concentrations of the test substance were measured in the vicinity of the breathing zone. In preliminary studies, it was found that the respirability of iprovalicarb dust was unsatisfactory, so the test material was micronized. MMAD, NMAD, and GSD were measured.

There were no mortalities and no clinical signs of treatment-related effects in any of the animals. There were no gross pathological abnormalities evident at necropsy.

Respirability was reported to be 44 and 29% for both sexes in the 505 and 4977 mg/m^3 groups respectively. Results of the analysis of the particle-size distributions in the dust in the breathing zone indicated that the inhalable mass fraction was comparatively small (29% ≤ 3µm aerodynamic diameter).

The acute inhalation LC_{50} for in the rat > 4977 mg/m^3 air. The low toxicity may be attributed to the specific properties of the substance and not just to the poor respirability, as the data generated in this study clearly show that iprovalicarbdoes not cause any damage to the respiratory tract. There is no classification for acute inhalation toxicity in the rat.

4.2.1.3 Acute toxicity: dermal

Iprovalicarb (98.4% purity) was mixed to a paste with Cremophor EL 2% v/v in physiological saline solution, and applied to the intact dorsal skin of two groups of (5) male and (5) female rats (Bor:WISW [SPF-Cpb]) at a single dose of 5000 mg/kg bw. After a 24 hour exposure period, under occlusion, the dressing was removed and the treated skin site was cleaned with soap and water.

There were no mortalities. No clinical signs or skin changes were observed. There were no gross pathological changes observed at necropsy.

The acute dermal LD_{50} > 5000 mg/kg bw.

4.2.1.4 Acute toxicity: other routes

Iprovalicarb (98.4% purity), formulated in physiological saline solution with 2% v/v Cremophor, was administered intraperitoneally by injection syringe to 5 males and 5 females

each at doses of 50, 200 and 500 mg/kg bw. The animals used were Wistar rats of the strain Bor:WISW (Spf-Cpb). Animals were sacrificed and necropsied after a 14 day observation period.

There were no mortalities during the course of the study. Clinical signs which were observed included vocalization on touch, apathy, spastic gait, staggering gait and soft faeces, and these were seen in all animals in the middle and high dose groups. All these signs were reversible within the post-treatment period. There were some indications of irritation of the abdominal cavity as a result of the i.p. administration e.g. ovaries surrounded by vesicula containing fluid, fusing of liver and diaphragm, swollen liver, white patch on upper liver lobe, substance residue on liver. Apart from this, no other test article related gross pathological changes were observed.

The acute i.p. LD_{50} > 500 mg/kg bw.

4.2.2 Human information

Not available.

4.2.3 Summary and discussion of acute toxicity

The acute toxicity of iprovalicarb was tested *via* the oral (rats and mice), dermal (rat), inhalation (rat) and intraperitoneal (rat) routes. The acute toxicity of iprovalicarb, regardless of route of exposure, was very low. There were no clinical signs observed, except in the intraperitoneal study, where minimal non-specific effects were noted.

There was no evidence of any sex-specific sensitivity in any of the studies.

In conclusion, there are no indications for classification under the CLP Regulation (EC) No. 1272/2008.

4.2.4 Comparison with criteria

Acute oral toxicity in the rat: No classification is proposed according to the CLP Regulation (EC) No. 1272/2008, where the cut-off value for Acute Tox. 4 (2000 mg/kg bw) has been exceeded. The LD_{50} in both rats and mice was > 5000 mg/kg bw.

Acute dermal toxicity in the rat: No classification is proposed according to the CLP Regulation (EC) No. 1272/2008, where the cut-off value for Acute Tox. 4 (2000 mg/kg bw) has been exceeded. The dermal LD_{50} in rats was > 5000 mg/kg bw.

Acute inhalation toxicity in the rat: No classification is proposed according to the CLP Regulation (EC) No. 1272/2008, where the cut-off value for Acute Tox. 4 (5 mg/l, dust or mist) has been exceeded. There were no deaths recorded at the limit dose of 4977 mg/m^3.

4.2.5 Conclusions on classification and labelling

No classification for acute toxicity *via* oral, dermal and inhalation routes.

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of iprovalicarb as acute toxicity for any of the three routes of exposure because two oral studies (one in rat and one in mouse) yielded LD_{50} > 5000 mg/kg bw; one dermal study in rat yielded LD_{50} > 5000 mg/kg bw and one inhalation study yielded LC_{50} > 4977 mg/m^3. All these four studies were conducted under the relevant OECD test guidelines (TG).

Comments received during public consultation

Two different member state competent authorities (MSCAs) supported the DS's proposal for no classification of iprovalicarb in regard to acute toxicity.

Assessment and comparison with the classification criteria

The table 1 summarises the main findings reported by the CLH dossier in the available acute toxicity studies.

Table 1: *Summary of the animal studies on acute toxicity studies with iprovalicarb***.**

According to the CLH dossier all studies were conducted under the relevant OECD TG.

Study	Dose level	Results	Reference
OECD TG 401, GLP Oral (oesophageal tube) Bor: WISW [SPF Cpb] rats Male/female 5 animals/sex Vehicle: Cremophor 2% (v/v) in water	98.4% iprovalicarb 5000 mg/kg bw The administration achieved by two separate deliveries of 20 ml/kg bw each, at approximately 6 hour intervals	No mortalities No indications of treatment-related systemic toxicity There were no effects on animal body weights LD_{50} > 5000 mg/kg bw for both sexes	Bomann, 1993; 22110 RAR B.6.2.1.1
OECD TG 401, GLP Oral (oesophageal tube) Bor: NMRI [SPF-Han] mice Male/female 5 animals/sex	98.4% iprovalicarb 5000 mg/kg bw The administration achieved by two separate deliveries of 20 ml/kg bw each, at approximately 6 hour intervals	No mortalities No indications of treatment-related systemic toxicity There were no effects on animal body weights No gross pathological changes	Bomann, 1993; 22108 RAR B.6.2.1.2

Vehicle: Cremophor 2% (v/v) in water		LD_{50} > 5000 mg/kg bw for both sexes	
OECD TG 402, GLP Dermal Bor:WISW rats Male/female 5 animals/sex Vehicle: 2% Cremophor in physiological saline	98.4% iprovalicarb 5000 mg/kg bw After 24 hours of occlusive exposure the treated skin area was cleaned with soap and water	No mortalities No clinical signs or skin changes No gross pathological changes LD_{50} > 5000 mg/kg bw for both sexes	Bomann, 1993 Study No. T4041135 RAR B.6.2.2
OECD TG 403, GLP Inhalation Bor:WISW rats Males/females 5 animals/sex/group	97.6% iprovalicarb Head/nose only Dust: 0, 505, and 4977 mg/m^3 air for 4 hours Concentrations of the test substance were measured in the vicinity of the breathing zone Respirability was reported to be 44 and 29% for both sexes in the 505 and 4977 mg/m^3 groups, respectively Inhalable mass fraction was comparatively small (29% ≤ 3 µm aerodynamic diameter)	No mortalities No clinical signs of treatment-related effects No gross pathological abnormalities No damage to the respiratory tract. LC_{50} > 4977 mg/m^3 air for both sexes	Pauluhn, 1993 Study No. T9071167 RAR B.6.2.3
Intraperitoneal Bor:WISW (Spf-Cpb) rats Males/females 5 animals/sex/group Vehicle: 2% Cremophor in physiological saline	98.4% iprovalicarb 50, 200 and 500 mg/kg bw Animals were sacrificed after 14 days	No mortalities Clinical signs (reversible) in middle and high group: vocalization on touch, apathy, spastic gait, staggering gait and soft faeces Some indications of irritation of the abdominal cavity as a result of the i.p. administration e.g. ovaries surrounded by	Bomann, 1993 Study No. T3041134 RAR B.6.2.7

		vesicula containing fluid, fusing of liver and diaphragm, swollen liver, white patch on upper liver lobe, substance residue on liver No pathological changes LD_{50} > 500 mg/kg bw for both sexes

Comparison with the criteria

An unusual aspect of the two oral studies is that the dose was administered in two separate deliveries 6 hours apart and it is therefore questionable whether the animals received a single dose of 5000 mg/kg bw, rather two of 2500 mg/kg bw. Nevertheless, mortalities were not observed in either of the oral studies (one in rat and one in mouse) and therefore, independently whether the dose can be considered as 2500 or 5000 mg/kg bw the LD_{50} would always be higher than 2000 mg/kg bw (the highest dose to be classified as acute toxicity category 4).

The results of the dermal acute toxicity study yielded LC_{50} higher than 2000 mg/kg bw (the highest dose to be classified as acute toxicity category 4).

In the same way, the results of the inhalation acute toxicity study and yielded LC_{50} higher than 5000 mg/m^3 (the highest dose to be classified as acute toxicity category 4). RAC also notes that the study using peritoneal route of exposure also revealed low acute toxicity of iprovalicarb.

In conclusion, the DS's proposal for **no classification of iprovalicarb in regard to acute oral, dermal and inhalation toxicity** is supported by RAC.

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

No signs of toxicity *via* routes tested.

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

Not required.

4.3.2 Comparison with criteria

Not required.

4.3.3 Conclusions on classification and labelling

No classification

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

Summary of the Dossier Submitter's proposal

The DS proposed no classification for STOT SE since no signs of toxicity were reported on the acute toxicity studies by the oral, dermal or inhalation routes.

Comments received during public consultation

One MSCA supported the DS's proposal for no classification of iprovalicarb regarding STOT SE.

Assessment and comparison with the classification criteria

No signs of toxicity were reported in the acute oral, dermal and inhalation toxicity studies and therefore RAC supports the DS proposal for **no classification of iprovalicarb for STOT SE**.

4.4 Irritation

4.4.1 Skin irritation

Table 12: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation rabbit (NZW) Vehicle: moistened with PEG Iprovalicarb: 98.4% pure	Non irritant	Guideline study. No sign of any irritation at any time point	Krötlinger, P. 1992 Study No. T2041197 RAR B6.2.4/5

4.4.1.1 Non-human information

In the dermal irritation study, 500 mg of pulverised iprovalicarb(98.4% purity), moistened with polyethylene glycol, was placed on a hypoallergenic patch and applied under a semi-occlusive dressing to the shaven, intact dorso-lateral area of the trunk of 3 adult (2 female; 1 male) albino rabbits (HC:NZW). After an exposure period of four hours, the dressing and patches were removed. The exposed skin areas were carefully washed with water. Dermal irritation was

scored (as by Draize) for erythema/eschar and oedema formation. This was recorded at exposure termination and at 24, 48 and 72 hours post-application.

There were no signs of irritation at any of the observation times, and there were no signs of any systemic toxicity in any of the treated animals.

4.4.1.2 Human information

None available.

4.4.1.3 Summary and discussion of skin irritation

No irritation seen in a guideline dermal irritation study.

4.4.1.4 Comparison with criteria

Acute dermal irritation in the rabbit: No classification is proposed according to the CLP Regulation (EC) No. 1272/2008, where the major criterion for the irritant category is that at least 2 of 3 tested animals have a mean score of $\geq 2.3 \leq 4.0$. All animals were negative with respect to dermal irritation for iprovalicarb.

4.4.1.5 Conclusions on classification and labelling

No classification.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of iprovalicarb for skin irritation on the basis of a skin irritation rabbit study showing no signs of irritation 24, 48 and 72 hours post-application.

Comments received during public consultation

Two different MSCAs supported the DS's proposal for no classification of iprovalicarb regarding skin irritation.

Assessment and comparison with the classification criteria

Table 2 summarises the results of the only skin irritation study available.

Table 2: *Summary of the animal study on skin corrosion/irritation with iprovalicarb***.**

Study	Dose level	Results	Reference
OECD TG 404, GLP HC:NZW albino rabbits	500 mg 98.4% iprovalicarb moistened with vehicle Semi-occlusive	No sign of any irritation at any time point (24, 48 and 72 hours post-application)	Krötlinger, 1992 Study No. T2041197 RAR

2 females, 1 male Vehicle: polyethylene glycol	dressing Exposure period: 4 hours and afterwards the exposed skin areas were carefully washed with water.	No signs of any systemic toxicity in any of the treated animals Non-irritant	B6.2.4/5

All animals gave negative results regarding dermal irritation. Draize scores were lower than 2.3 and 4.0 for erythema and for oedema respectively therefore, the classification criteria is not met. Thus, RAC supports the DS proposal for **no classification of iprovalicarb regarding skin irritation.**

4.4.2 Eye irritation

Table 13: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation rabbit (NZW)	No classification	Minimal irritation at 24 hours in one animal, totally reversible by 48 hours	Krötlinger, P. 1992 Study No. T2041197 RAR B.6.2.4/5

4.4.2.1 Non-human information

In the eye irritation/corrosion study, a volume of 100 µl (approx. 22 mg) of the pulverised test substance (iprovalicarb- 98.4% purity) was applied to the conjunctival sac of one eye of each of three adult (2 female; 1 male) rabbits (HC:NZW). The test substance was rinsed from the treated eye with normal saline. Eye irritation was scored (as by Draize) and recorded at 1, 24, 48 and 72 hours and 7, 14 and 21 days.

Minimal irritation of the conjunctivae was observed in one animal at 24 hours only (Draize score 1), and this did not persist. Otherwise, there were no signs of treatment-related effects in any of the animals.

4.4.2.2 Human information

None available.

4.4.2.3 Summary and discussion of eye irritation

Minimal reversible irritation was seen in a guideline study, which did not meet the criteria for classification.

4.4.2.4 Comparison with criteria

Acute eye irritation in the rabbit: No classification according to the CLP Regulation (EC) No. 1272/2008 is proposed, as EU trigger values were not exceeded in any animal (a positive response in at least 2 of 3 tested animals was not observed; the criteria for a positive response in a single animal are mean gradings for conjunctiva – redness (or chemosis): ≥ 2.0; iritis: ≥ 1; corneal opacity: ≥ 1).

4.4.2.5 Conclusions on classification and labelling

No classification.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

DS proposed no classification of iprovalicarb for eye corrosion on the basis of an eye irritation rabbit study showing reversible effects (Draize score 1) in one animal 24 hours post-application.

Comments received during public consultation

Two different MSCAs supported the DS's proposal for no classification of iprovalicarb regarding eye irritation.

Assessment and comparison with the classification criteria

The table 3 summarises the results of the only eye irritation study available.

Table 3: *Summary of the animal study on eye corrosion/irritation with iprovalicarb*

Study	Dose level	Results	Reference
OECD TG 405, GLP HC:NZW albino rabbits 2 females, 1 male Vehicle: saline Eye irritation was scored (as by Draize) and recorded at 1, 24, 48 and 72 hours and 7, 14 and 21 days.	100 µl containing approximately 22 mg 98.4% iprovalicarb was applied to the conjunctival sac of one eye The test substance was rinsed from the treated eye with normal saline	Minimal irritation of the conjunctivae was observed in one animal at 24 hours only (Draize score 1) and this did not persist. No signs of treatment-related effects in any of the animals	Krötlinger, 1992 Study No. T2041197 RAR B.6.2.4/5

Trigger values for classification were not exceeded since the slight reported effects were reversible and Draize scores for conjunctiva-redness, iritis and corneal opacity were

always lower than 2, 1 and 1, respectively. Therefore, RAC supports the DS's proposal for **no classification of iprovalicarb for eye irritation**.

4.4.3 Respiratory tract irritation

No data

4.4.3.1 Non-human information

4.4.3.2 Human information

4.4.3.3 Summary and discussion of respiratory tract irritation

4.4.3.4 Comparison with criteria

4.4.3.5 Conclusions on classification and labelling

4.5 Corrosivity

No corrosivity was seen in the guideline skin and eye irritancy study.

Table 14: Summary table of relevant corrosivity studies

Method	Results	Remarks	Reference
-	-	-	-

4.5.1 Non-human information

Not corrosive in the guideline rat study.

4.5.2 Human information

None available.

4.5.3 Summary and discussion of corrosivity

Not required.

4.5.4 Comparison with criteria

Not required.

4.5.5 Conclusions on classification and labelling

No classification.

4.6 Sensitisation

4.6.1 Skin sensititsation

Table 15: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
M &K skin sensitisation (Bor:DHPW guinea pigs) Vehicle: 2% Cremophor in physiological saline AS purity: > 98.7%	Negative	no signs of irritation after challenge	Kolb, J. 1992 Study No. T1041178

4.6.1.1 Non-human information

The test substance (iprovalicarb; 98.7 - 99.4% purity) was formulated with 2% v/v Cremophor ® EL. Formulations containing more than 25% test substance could not be produced. The doses for the induction and challenge exposures were selected on the basis of range-finding test results.

Intradermal injections (2 x 0.1 ml) were administered to three sites along each side of the spine. The treated group received 1:1 Freund's adjuvant in saline, 5% test substance in saline with 2% v/v Cremophor® and 5% test substance in 1:1 Freund's adjuvant and saline with 2% Cremophor®. The controls received the same preparations withoutiprovalicarb. Animals were subsequently observed for 7 days.

Topical induction was performed using 0.5 ml of 25% formulation on an application site which had been shaved and painted with a 10% formulation of sodium lauryl sulfate in liquid paraffin one day in advance. Controls received saline with 2% Cremophor® only.

Topical challenge was performed three weeks after intradermal induction. In the challenge, 0.5 ml of a 25% formulation of the test substance, and secondly, a 12% formulation were placed on the left flanks of the animals in the test substance and control group and fixed to the skin for 24 hours with adhesive tape. Test substance residues were removed at the end of the exposure period, and skin reactions were graded at 48 and 72 hours after initiation of the challenges.

Induction: The intradermal induction concentration of 5% iprovalicarb was chosen on the basis of the signs observed in animals in the pilot test (wheals and reddening at 1 - 5%). In the main study, no effects of treatment were reported.

The dermal induction concentration (25%iprovalicarb) was again selected on the basis of the pre-study which showed no evidence of any irritation at this application rate. However, this was the maximum producible concentration, so treatment areas of the animals were pre-stimulated with sodium lauryl sulfate prior to the second induction.

Challenge: After dermal challenge treatment with 12 and 25%, no dermal reactions occurred in either the test substance or the control group.

The test substance was non-sensitising.

4.6.1.2 Human information

None available.

4.6.1.3 Summary and discussion of skin sensitisation

Iprovalicarb was not sensitising in a guideline M&K study.

4.6.1.4 Comparison with criteria

Skin sensitisation: No classification is proposed according to the CLP Regulation (EC) No. 1272/2008, where the major criterion for a significant skin sensitising effect is erythema in ≥ 30% of the test animals. There was no evidence of any dermal reaction following a challange with 12% and 25% test substance formulations in an M&K guinea pig sensitisation study.

4.6.1.5 Conclusions on classification and labelling

No classification required.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

DS proposed no classification of iprovalicarb for skin sensitisation in a Magnusson & Kligman study.

Comments received during public consultation

Two different MSCAs supported the DS's proposal for no classification of iprovalicarb as skin sensitisation.

Assessment and comparison with the classification criteria

The table 4 summarises the results of the only skin sensitisation study available.

Table 4: *Summary of the animal study on skin sensitisation with iprovalicarb*

Study	Dose level	Results	Reference
OECD TG 406, GLP Magnusson & Kligman skin sensitisation Bor:DHPW female guinea pigs 20 treated animals 10 control animals Vehicle: 2% Cremophor in physiological saline Formulations containing more than 25% test substance could not be produced 98.7-99.4% iprovalicarb	Treated group: 1:1 Freund's adjuvant in saline, 5% iprovalicarb in vehicle and 5% iprovalicarb in 1:1 Freund's adjuvant and vehicle. Controls groups: the same	No signs of irritation after challenge	Kolb, 1992 Study No. T1041178

Intradermal induction: 2 x 0.1 ml injections to three sites along each side of the spine. Topical induction: 7 days after intradermal induction 0.5 ml of 25% formulation on an application site which had been shaved and painted with a 10% formulation of sodium lauryl sulphate in liquid paraffin one day in advance. Challenge: 0.5 ml of a 25% formulation of the test substance, and secondly a 12% formulation were placed on the left flanks of the animals in the test substance and control group and fixed to the skin for 24 hours with adhesive tape.	preparations without iprovalicarb

RAC notes that it is unknown whether positive controls were present in this test. Nevertheless, RAC also notes the absence of evidence for any dermal reaction following a challenge with 12% and 25% test substance formulations in a Magnusson & Kligman skin sensitisation study. Thus, RAC supports the DS's proposal for **no classification of iprovalicarb regarding skin sensitisation.**

4.6.2 Respiratory sensitisation

No data.

Table 16: Summary table of relevant respiratory sensitisation studies

Method	Results	Remarks	Reference
-			

4.6.2.1 Non-human information

None available.

4.6.2.2 Human information

None available.

4.6.2.3 Summary and discussion of respiratory sensitisation

Not required.

4.6.2.4 Comparison with criteria

Not required.

4.6.2.5 Conclusions on classification and labelling

Not required.

4.7 Repeated dose toxicity

Table 17: Summary table of relevant repeated dose toxicity studies

Method	Results	CLP GV	Reference
Rat studies			
28 day feeding study, Wistar rat AS: 99.4%. 0, 2000, 6000, 20000 (195.8/198.7, 579.3/572.8, 1973.9/1934.4) Iprovalicarb: 99.4%	**20000 ppm:** Mortality/clinical signs: No mortalities and no clinical signs. Body weight /food consumption: Slight, transient reduced body weight gain (♂). Haematology: No treatment-related findings. Blood chemistry: Stat sig ↑ALP, (♂) and ↑cholesterol (♂♀), triglycerides (♀), stat sig ↓creatinine, urea (♂). Stat sig. ↑O-demethylase and P-450 (♂♀). Organs weight: Stat sig. ↑relative (♂♀) and absolute (♀) liver weight. Stat sig ↓ absolute and relative pituitary weight (♂). Pathology: No histological findings. **6000 ppm:** Blood chemistry: Stat sig. ↑cholesterol and triglycerides (♀). Stat sig ↓creatinine and urea (♂). Stat sig. ↑O-demethylase (♂♀) and P-450 (♀). Organs weight: Stat sig. ↑relative and absolute liver weight(♀). Slight stat sig ↓ absolute ((♂♀) and relative pituitary weight (♂). LOAEL: 6000 ppm (579.3/572.8)	≤ 300 mg/kg No classification	Bomann, W., 1995 RAR B.6.3.1.1
13-week feeding study with 4-week recovery (high dose group). Wistar rat Iprovalicarb: 98.1 - 98.7% 0, 1250, 5000, 20000 (87.4/133.9, 372.7/561.4, 1524/2585.9).	**20000 ppm:** Mortality/clinical signs: None Body weight/food consumption: Slight reduction in weight gain (♂♀) –recovered. ↑food intake (♀) also ↑ during the recovery period. Haematology: No effects Blood chemistry: Slight non stat sig. ↑ALP, (♂). Stat sig. ↓triglycerides (♂). Stat sig. ↑O-demethylase and P-450 (♂♀). Organs weight: Stat sig. ↑ absolute (♀). and relative liver weight (♂/♀). Normal after recovery period. Pathology: No findings **5000 ppm:** Organs weight: Stat sig. ↑ relative liver weight (♂/♀). LOAEL: 5000 ppm (372.7/561.4 mg/kg bw (♂/♀)).	≤ 100 mg/kg bw No classification	Schladt, L., Watta-Gebert, B., and Rinke, M., 1996 RAR B.6.3.2.1
Mouse studies			

13 week feeding study, B6C3F1 mouse (98.1 - 98.7%) 0, 280, 1400, 7000, 14000 (63/125, 325/696.5, 1724.6/3599.5, 3473/6869)	**14000 ppm:** Mortality/clinical signs: None Body weight/food consumption: No effect. Water intake: ↑intake (♂/♀). Haematology: Slight stat sig ↑ MCV and MCH (♂). Blood chemistry: marginal ↑ cholesterol (♂/♀). Organs weight: Stat sig. ↑ absolute (♂). and relative liver weight (♂/♀) and ↓ absolute and relative kidney weight (♂). Pathology: No treatment-related findings. **7000 ppm:** Organ weight: Stat sig. ↑ relative liver weight (♀) Water intake: ↑intake (♂/♀). LOAEL: 7000 ppm (1724.6/3599.5 mg/kg bw (♂/♀).	≤ 100 mg/kg No classification	Watta-Gebert, B., and Popp, A., 1996 RAR B.6.3.2.2
Dog studies			
4 week feeding study, beagle AS: 98.1% pure 0, 100, 1000, 10000, 50000 (3/3.4, 31.5/35, 280/269.5, 1322/1164.5) Iprovalicarb 98.1%	**50000 ppm:** Mortality and clinical signs of toxicity: No mortalities and no clinical signs. Body weight/food consumption: Reduced body weight (♂♀). Haematology: Slight ↑ in APTT (♂) Blood chemistry: Stat sig ↑ AP (♂♀). ↓cholesterol (stat sig. ♀). Urinalysis: ↓Urine volume, pH, and electrolytes ((♂♀) Organs weight modifications: ↑relative and absolute liver weight (♂♀). Pathology: enlarged hepatocytes with a ground-glass appearance. **10000 ppm:** Mortality and clinical signs of toxicity: No mortalities and no clinical signs. Body weight/food consumption: Reduced body weight (♀). Haematology: Slight ↑ in APTT (♂). Blood chemistry: Stat sig ↑ AP (♀). ↓cholesterol (♂) Organs weight modifications: ↑relative and absolute liver weight (♂♀). Pathology: Enlarged hepatocytes with a ground-glass appearance. **1000 ppm:** Pathology: **Mild/minimal** enlarged hepatocytes with a ground-glass appearance. LOAEL: 1000 ppm (31.5 mg/kg bw)	≤ 300 mg/kg	Porter, M.C., Jasty, V., Grosso, D.S., and Hartnagel Jr., R.E., 1993 RAR B.6.3.1.2

13 week feeding study. Purebred beagle (4/sex) Iprovalicarb: 95.8 - 98.5% pure. 0, 250, 2500, 50000 (9.1, 62.5, 1250) No statistical analysis. Data from sexes pooled.	**50000 ppm:** Mortality/clinical signs: None Body weight/food consumption: Slightly reduced weight gain and food consumption. Haematology: No effects Urinalysis: No effects. Blood chemistry: ↑↑AP, AST, ALT, GLDH, LDH. ↑ triglycerides. ↓Cholesterol, protein, albumin. ↑ N-DEM, O-DEM, P-450 Organs weight: ↑ Absolute and relative liver weight. ↓ testes and prostate weight. ↓ thymus weight. Pathology: Discolouration and distinct lobulation Microscopy: Hypertrophy, vacuolation, focal necrosis/single cell necrosis, iron within periortal hepatocytes and Kupffer cells. Adnormal gallbladder content. **2500 ppm:** Mortality/clinical signs: None Body weight/food consumption: No effect Haematology: No effect Blood chemistry: ↑ AP, ↓ protein/albumin. ↑ triglycerides. ↑ N-DEM, O-DEM, P-450. Organs weight: ↑ Absolute and relative liver weight. Pathology: Discolouration and distinct lobulation. Microscopy: hepatocellular hypertrophy and multilamellar bodies. **250 ppm (9.1 mg/kg bw):** Organ weights: **Slightly** increased absolute and relative liver weights. LOAEL: not found	≤ 100 mg/kg	Vliegen, M. and Hartmann, E., 1995 RAR B.6.3.2.3

53 week feeding study, purebred beagle (4 males, 5 females)) AS: 98.7% pure 0, 80, 800, 8000 (2.6/2.68, 24.69/28, 256.86/262.41) No statistical analysis.	**8000 ppm:** Mortality/clinical signs: None Body weight/food consumption: Slight ↓ on body weight (♂) Haematology: No relevant treatment-related effects. Blood chemistry: ↑↑ALAT, AP, ASAT, GLDH, GGT (♂♀). ↓Albumin. ↑↑ N-DEM, O-DEM, P-450 (♂♀). Organs weight: ↑↑Absolute and relative liver weights (♂♀). Pathology: Hepatocellular hypertrophy, fatty change, ↑ iron storage. Focal necrosis and ↑binucleated hepatocytes. Fibrosis of liver parenchyma (3 animals) with marked nodular hyperplasia (1/3). ↑ Adhesive mucus (3/4) and pseudogland formation in gall bladder (2/4 ♂ and ♀) . Inactive prostrate, ↓spermatogenesis, epididymal aspermia (1/4 ♂) accompanied by other significant signs of ill health (emaciation, clinical chemistry, liver histopathology). **800 ppm:** Blood chemistry: ↑↑ALAT, AP. ↑↑ N-DEM, O-DEM, P-450 (♂♀). Organ weights: ↑↑Absolute and relative liver weights (♂♀). Pathology: Hepatocellular hypertrophy, fatty change, ↑ iron storage (3/5 ♀). ↑ Adhesive mucus (3/4). **80 ppm:** Clinical chemistry: ↑ N-DEM, O-DEM, P-450 (♂♀). Slight ↑AP and ALAT (♂) Organs: ↑Absolute and relative liver weights (♂). Periportal fatty change (1/4 ♂). Iron pigment in hepatocytes (1/5 ♀) LOAEL < 80 ppm (2.6 mg/kg bw)	≤ 12.5 mg/kg	Ruf, J. and Rinke, M., 1997 RAR B.6.3.3.1
Supplementary 28 day feeding study: liver enzyme induction. Beagle Iprovalicarb: 98.9% 0, 10, 20, 40, 80 (0.41, 0.77, 1.61, 3, 2.93)	Mortality/clinical signs: None Body weight/food consumption: No effects. Haematology: No effects. Blood chemistry: No effect on liver marker enzymes. Microsomal enzymes: Stat sig ↑ in N-demethylase, O-demethylase and P 450 from 40 ppm (♂♀) reversible after a 28 day recovery period.. Organs weight: No effect on liver weight. NOEL for enzyme induction: 20 ppm (0.77 mg/kg)	≤ 300 mg/kg No classification	Wetzig, H. and Rinke, M., 1997 RAR B.6.3.1.3

5 day inhalation study, Wistar rat Iprovalicarb: 97.6% pure 0, 20.6, 102.9, 504.4 mg/m^3 High dose = max. technically feasible concentration MMAD: approx. 1.3-1.8 µm GSD: 1.9-2.5 Particle size at least 80% <3 µm	**504.4 mg/m^3** Mortality/ clinical signs: None Body weight/food consumption: None Haematology: None Blood chemistry: None Organs weight: No effects LOEC > 504.4 mg/m^3	≤ 0.6 mg/m^3 No classification	Pauluhn, J., 1993 RAR B.6.3.4.1
4 week subacute dermal toxicity study, HC:NZW rabbits Iprovalicarb: 95.8% pure 0, 1000 mg/kg bw Limit dose	Mortality/clinical signs: None Body weight/food consumption: None Haematology: None Blood chemistry: None Organs weight: None LOAEL: > 1000 mg/kg bw	≤ 600 mg/kg No classification	Vohr, H.-W., and Geiß, V., 1995 RAR B.6.3.4.2

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Study 1: 28 day feeding study, Wistar rat: RAR B.6.3.1.1.

No test substance-related mortalities, ophthalmic observations or clinical signs were observed during the study. In the high dose male group, transient, slight retardation of the body weight development was noted in week 1. A slightly elevated erythrocyte count, haemoglobin content and haematocrit reading were seen in the high dose females. No comparable effect was seen in the male animals, and this was therefore treated as an incidental finding. Over all, the haematological observations gave no evidence of any test substance-related effects on the blood cell population.

Clinical chemistry findings were as follows; statistically significant increases in the alkaline phosphatase activity and cholesterol level in the male high-dose animals; cholesterol and triglyceride levels were significantly increased from 6000 ppm in females; reduced creatinine and urea levels in males from 6000 ppm. Other slight variations were considered incidental. In addition, O-demethylase was increased in males at from 6000 ppm, and cytochrome P-450 in the high dose only. In the females, both O-demethylase and P-450 activity were increased from ≥ 6000 ppm.

No significant effects were noted in the urinalysis or during necropsy at study termination.

Males exhibited slightly reduced pituitary weights in the mid-dose group and above and slightly elevated thyroid/depressed thymus weights in the high-dose group. Absolute liver weights were elevated in the females in the mid-dose group and above. The increase in liver weights was considered treatment-related, which is supported by increased P-450 activity in females at ≥ 6000 ppm, and increased O-demethylase activity in both males and females at ≥6000 ppm. In the context of all the data reported, the liver findings may represent marginal adverse effects at the LOAEL of approximately 572.8 mg/kg w.

Table 18: Organ weight changes.

Findings (wk 4)		0 ppm	2000 ppm	6000 ppm	20000 ppm
Abs. liver wt. [mg]	m	11941	11396	11916	12591
	f	6834	7207	7399*	7977**
Rel. liver wt. [mg/100 g]	m	4561	4532	4713	5052**
	f	4092	4439**	4637**	5062**
Abs. pituitary wt. [mg]	m	7	6	5**	5**
	f	7	7	5*	6
Rel. pituitary wt. [mg/100 g]	m	3	2	2**	2*
	f	4	4	3	4
Abs. thymus wt. [mg]	m	554	540	499	484*
	f	347	340	346	353
Abs. thyroid wt. [mg]	m	5	5	6	6*
	f	3	5	4	4
Rel. thyroid wt. [mg/100 g]	m	2	2	2*	3*
	f	2	3	2	3
** (**)Significantly different from control at the 0.05 (0.01) level (U-test)*					

Study 2: 13-week feeding study; **Wistar rats.** RAR B.6.3.2.1

There were no treatment-related mortalities, or clinical signs of toxicity. Body weights were slightly reduced in the high dose animals, but recovered by the end of the recovery period. Elevated food intake was noted at the high dose in females (17% above controls) and still elevated after recovery (19%). Slight alterations in blood parameters appeared incidental and no effects were seen after the recovery period. Slight alterations in alkaline phosphatase (AP), cholesterol, triglycerides at the high dose were reversed after recovery. Evidence of liver induction included increased n-demethylase, o-demethylase and Cyt P-450 from 5000 ppm. No specific findings were noted at necropsy. Organ weight determinations revealed increased absolute liver and heart weights in females at 20000 ppm (14%) and increased relative liver weights in both males and females at 5000 ppm (9% and 8% respectively) and 20000 ppm (17% and 22%, respectively), which reversed during the recovery period. There were no histopathological findings.

Table 19: Organ weight changes.

Findings wk 13 (wk 17)		0 ppm (+ 4-wk recovery)	1250 ppm	5000 ppm	20000 ppm	20000 ppm + 4-wk recovery
Abs. Liver wt [g]	m	14.8 (15.4)	15.2	16.5	16.5	(14.5)
	f	8.0 (7.5)	7.9	8.5	9.1**	(7.9)
Rel. liver wt. [mg/100 g bw]	m	3392 (3248)	3493	3697*	3958**	(3258)
	f	3393 (2962)	3322	3678*	4147**	(3303)
Abs. Heart wt [mg]	m	1247 (1443)	1265	1385	1222	(1440)
	f	874 (896)	861	823	788**	(843)
** (**)Significantly different from control at the 0.05 (0.01) level (U-test)*						

Study 3: <u>13 week</u> feeding study; B6C3F1 mouse. RAR B.6.3.2.2.

There were no mortalities or clinical signs and no treatment related effects on body weights or food consumption. Water consumption was increased in animals from 7000 ppm, but was without clinical chemistry or histological correlate. Slight alterations in haematological parameters were noted at the high dose only. Cholesterol was slightly elevated in males from 1400 and 7000 ppm but not at 14000 ppm groups, and in females beginning at 7000 ppm,

compared with control animals (increased cholesterol levels were also noted in the 13-week rat study). Effects on cholesterol levels were marginal and therefore not considered to be indicative of an adverse effect.

Table 20: Clinical findings

Findings		0 ppm	280 ppm	1400 ppm	7000 ppm	14000 ppm
RBC [10^{12}/l]	m	9.70	9.62	9.58	9.52	9.31*
	f	9.36	9.34	9.29	9.24	9.03
Haematocrit [l/l]	m	0.448	0.443	0.447	0.448	0.443
	f	0.436	0.435	0.432	0.429	0.420
MCV [fl]	m	46.2	46.0	46.6	47.1**	47.6**
	f	46.6	46.5	46.5	46.5	46.6
MCH	m	15.5	15.6	15.6	15.6	15.9**
	f	15.8	15.8	15.9	16.1	16.2
Cholesterol [mmol/l]	m	3.20	3.23	3.55**	3.53*	3.13
	f	2.58	2.75	2.74	2.82*	2.87*
** (**)Significantly different from control at the 0.05 (0.01) level (Dunnett's test)*						

Any findings at necropsy were isolated and unrelated to test substance treatment. There was a significant increase in absolute liver weights in group 14000 ppm (males 18%; females 11%) and in relative liver weights in males at this dose (17%) and in females at 7000 and 14000 ppm (11% and 13%, respectively). Males in the 14000 ppm group exhibited a slight but significant reduction in absolute (10%) and relative (11%) kidney weights. However, there was no histopathological evidence of any treatment-related changes to the liver.

The LOAEL was 7000 ppm (1724.6/3599.5 mg/kg bw) on the basis of slight liver effects (increased weight, increased cholesterol levels), kidney (decreased weight, increased water intake), and changed haematological parameter (MCV).

Study 4: <u>4 week</u> feeding study, beagle: RAR B.6.3.1.2

No deaths occurred and all animals appeared in good health through scheduled termination after 4 weeks. There were no clinical observations which were considered test article-related. Emesis, soft stools/diarrhoea were noted sporadically across all groups. The two higher dose levels had an adverse effect on body weight as all animals at 50000 ppm and both females at 10000 ppm lost body weight during the 4-week course of the study. Food consumption was reduced for both sexes at dietary concentrations of 10000 and 50000 ppm.

In general, serum liver enzymes were increased at 50000 ppm after 4 weeks. Alkaline phosphatase (AP) was the most sensitive marker, as both dose- and time-related increases were observed for this enzyme. Very subtle AP increases in 1 dog of each sex in the 1000 ppm group suggested early hepatic involvement at that dose. There was a slight dose-related decrease in APTT (activated partial thromboplastin time, statistically significant) for 50000 ppm males by week 2, and for 10000 ppm dosed males by week 4.

Cholesterol was significantly decreased at 50000 ppm in females at weeks 2 and 4 and generally, but not significantly, reduced from 10000 ppm in males.

Urine volume, pH, and electrolytes were generally decreased in animals of both sexes from 10000 ppm. Reduced levels of electrolytes may be due to the decreased urine volume. All

these findings together indicate some test substance-related kidney effect at high doses. There were no findings at necropsy.

Absolute and relative-liver weights were increased for males (not statistically significant) and markedly for females from 10000 ppm. Although not significantly significant, the effects were clearly biologically relevant.

The liver as target organ was confirmed by microscopic changes seen in the livers of dogs from 1000 ppm. Hepatocytes appeared large and contained cytoplasm having a ground-glass appearance, indicative of increased proliferation of the smooth endoplasmic reticulum. No effects were seen in at 100 ppm. The LOAEL was 100 ppm on the basis of a biologically significant increase in absolute and relative weight coincident with altered histopathology indicative of increased metabolic activity.

Study 5: 13 week feeding study in beagle dogs (4/sex): RAR B6.3.2.3

One high dose female was sacrificed prematurely, due to its emaciated state. There were no other mortalities and no clinical signs of toxicity. Lower body weight gains were observed in high dose animals as a result of the reduced food consumption. No relevant changes in any of the haematological parameters measured were observed in treatment groups compared with the control group. Urinalysis gave no indications of a test substance-related effect. Distinct increases in the activities of the liver enzymes ALAT, ASAT, and GLDH were observed in dogs of the high dose group. LDH activity was slightly increased at the end of the treatment period (week 13). AP activity was slightly elevated at 2500 ppm and markedly elevated at 50000 ppm. Cholesterol was significantly (no statistical analysis) decreased in dogs of the high dose. Protein, especially albumin, was significantly decreased from ≥ 2500 ppm and might be the result of reduced synthesis in the liver. Microsomal liver enzymes (N-demethylase and O-demethylase) were induced by iprovalicarb treatment at 250 ppm and 2500 ppm. Cytochrome P-450 content and triglycerides were also increased.

Table 21: Relevant clinical chemistry findings.

Findings (wk 13)	**male + female**			
	0 ppm	**250 ppm**	**2500 ppm**	**50000 ppm**
AP [U/l]	192	217	↑↑345	↑↑759
ASAT [U/l]	16.2	13.8	12.8	↑↑41.0
ALAT [U/l]	16.7	↑26.6	↑23.9	↑↑272.8
GLDH [U/l]	1.5	1.0	1.2	↑↑77
LDH [U/l]	50	41	43	↑↑72
Cholesterol [mmol/l]	3.60	3.66	↓3.37	↓0.95
Protein [g/l]	62.2	58.5	↓55.5	↓48.2
Albumin [g/l]	35.5	32.0	↓29.4	↓20.6
Hepatic N-DEM [mU/g]	87.5	↑↑226.0	↑↑303.0	↑↑223.8
Hepatic O-DEM [mU/g]	23.2	↑↑44.9	↑↑58.2	↑38.0
Hepatic P-450 [nmol/g]	19.5	↑↑41.3	↑↑62.8	↑↑67.3
Hepatic TRIGL [µmol/g]	4.26	↑5.97	↑↑9.45	↑↑6.22

Data was not subjected to statistical analysis due to the limited number of animals per group

Absolute and relative liver weight was increased in the animals from ≥ 2500 ppm and slightly increased in mid-dose females and low dose males. Macroscopically, the testes of two males and prostate of all males of the high dose group were reduced. The thymus of three males of the high dose and one dog of the low dose were reduced.

Table 22: Relevant histopathological findings

Findings (wk 13)	**male + female**			
	0 ppm	**250 ppm**	**2500 ppm**	**50000 ppm**
Abs liver wt.	320	360	↑↑426	↑↑419
Rel. liver wt. [g/kg]	36	↑43	↑50	↑↑61
Abs. testes wt.	12.6	16.2	15.0	↓4.4
Rel. testes wt. [g/kg]	1	2	2	1
Abs. prostrate wt.	2.3	2.1	↓1.8	↓0.8
Abs. thymus wt.	8.0	8.4	7.6	↓3.4
Rel. thymus wt [g/kg]	1	1	1	0
Data was not subjected to statistical analysis due to the limited number of animals per group				

Macroscopic findings in the liver (discoloration, distinct lobulation) were present in male dogs of all dose groups and in one high dose female. Change in contents of the gallbladder occurred in higher frequency in animals at 50000 ppm. The most important treatment-related liver lesion identified at microscopy, was the occurrence of hepatocellular cytoplasmic change. Cytoplasmic change occurred in males and females of all treatment groups. At a dose level of 2500 ppm hepatocellular hypertrophy and multilamellar bodies were observed in one female each. Besides these liver findings, high dose animals exhibited cytoplasmic vacuolation, focal necrosis, single cell necrosis and iron containing pigments (haemosiderin) within periportal hepatocytes and Kupffer cells. The abnormal contents of the gallbladder correlated microscopically with oedemas and dilation of lymphatic vessels. The other histopathological findings of the high dose group (atrophy of the adipose tissue of subcutis and tongue, atrophy of the lymphatic tissues, serous atrophy of the bone marrow (femur/sternum), and retardation of the development of the male sexual organs) were considered to be associated with the poor physical condition of the dogs.

Table 23: Summary of relevant histological findings.

Findings (wk 13)		**0 ppm**	**250 ppm**	**2500 ppm**	**50000 ppm**
Liver, hepatocell. cytoplasmic change	**m**	0/4	3/4	3/4	4/4
	f	0/4	2/4 minimal	4/4 slight	3/4 moderate
Liver, hepatocell. vacuolation	**m**	0/4	0/4	0/4	1/4
	f	0/4	0/4	0/4	2/4 moderate or slight
Liver, hepatocell. hypertrophy	**m**	0/4	0/4	0/4	2/4
	f	0/4	0/4	1/4 minimal	3/4 moderate
Liver, multilamellar bodies	**m**	0/4	0/4	0/4	1/4
	f	0/4	0/4	1/4	2/4

Findings (wk 13)		0 ppm	250 ppm	2500 ppm	50000 ppm
Liver, focal necrosis	**m**	0/4	0/4	0/4	3/4
	f	0/4	1/4 moderate	0/4	1/4 moderate (m) slight (f)
Liver, single cell necrosis	**m**	0/4	0/4	0/4	4/4
	f	0/4	0/4	0/4	2/4 slight or moderate
Liver, iron pigment	**m**	0/4	0/4	0/4	2/4
in Kupffer cells	**f**	0/4	0/4	0/4	3/4
Liver, iron pigment	**m**	0/4	0/4	0/4	3/4
in periportal hepatocytes	**f**	0/4	0/4	0/4	1/4
Liver, granulocytic	**m**	0/4	0/4	1/4	3/4
infiltration	**f**	0/4	0/4	0/4	1/4
Gallbladder, abnorm. content	**m**	0/4	0/4	0/4	3/4
	f	0/4	0/4	0/4	2/3
Gallbladder, edema wall		0/4	1/4	1/4	4/4
		1/4	1/4	1/4	3/3
Gallbladder, dilated	**m**	0/4	1/4	1/4	4/4
lymphatic vessel	**f**	0/4	0/4	1/4	2/3
Testes, juvenile	**m**	1/4 no data	0/4	1/4 slight	4/4 slight to massive
Epididymides, juvenile	**m**	1/4 no data	0/4	1/4 slight	4/4 moderate to massive
Prostrate, juvenile	**m**	1/4 no data	1/4 no data	1/4 slight	4/4 marked to massive
Thymus, atrophy	**m**	0/4	0/4	0/4	4/4
	f	0/4	0/4	0/4	2/3
Bone marrow, serous atrophy	**m**	0/4	0/4	0/4	3/4
	f	0/4	0/4	0/4	1/4
Data was not subjected to statistical analysis due to the limited number of animals per group					

In conclusion, the liver was targeted with evidence of both adaptive and toxicological change from 2500 ppm with some evidence of adaptive change at the lowest dose level 250 ppm.

Study 6: <u>53 week</u> feeding study, purebred beagle. RAR B.6.3.3.1

No deaths occurred and all animals appeared in good health through scheduled termination. There were no clinical observations which were considered test article-related. Body weight in males was reduced at 8000 ppm. Food consumption was not reduced. Haematological parameters were not affected. Clinical-chemical investigations revealed increased activities of ALAT and AP at 800 ppm and above. In addition, at 8000 ppm, ASAT, GLDH, and GGT were increased. Plasma albumin values were decreased in 8000 ppm animals. There was a dose-dependent increase in N- and O-demethylase and Cyt P450 activities from 80 ppm and upwards. The triglyceride content in the liver was increased from 800 ppm and above. Urinalysis results revealed no abnormalities at dose levels up to and including 8000 ppm.

Absolute and relative liver weights were increased in mid-dose group males and above and in high-dose group females. A slight increase in the mean relative liver weight (15%) was also seen in males of the low-dose group, which was caused by marked increases in liver weight of two dogs (+27%), while the remaining two dogs showed liver weights that were within or even below control ranges. Increases in relative adrenal weight observed in high-dose males were not accompanied by any related clinical-chemical or histopathological findings. Taking into account the high standard deviation ranges observed in all treatment groups, this finding is considered incidental.

Table 24: Summary of histopathological change.

Selected findings		0 ppm	80 ppm	800 ppm	8000 ppm
LIVER					
Hepatocellular cytoplasmic change	**m**	0/4	0/4	4/4 (min-sli)[1]	4/4 (mod)
	f	0/4	0/4	4/5 (sli)	4/4 (mod)
Hepatocellular hypertrophy	**m**	0/4	0/4	4/4 (min)	4/4 (sli-mod)
	f	0/4	0/4	4/5 (min-sli)	4/4 (sli-mod)
Hepatocellular multilamellar inclusions	**m**	0/4	0/4	0/4	1/4 (min)
	f	0/4	0/4	0/5	3/4 (sli-mod)
Periportal fatty change	**m**	0/4	1/4 (sli)	3/4 (min-sli)	2/4 (mod)
	f	0/4	0/4	3/5 (min-sli)	1/4 (min)
Binucleated hepatocytes	**m**	0/4	0/4	0/4	3/4 (min-sli)
	f	0/4	0/4	0/5	2/4 (min-sli)
Focal necrosis	**m**	0/4	0/4	0/4	2/4 (min-sli)
	f	0/4	0/4	0/5	0/4
Single cell necrosis	**m**	1/4 (min)	0/4	0/4	1/4 (min)
	f	1/4 (min)	0/4	0/5	4/4 (min
Iron pigment in hepatocytes	**m**	1/4 (min)	0/4	0/4	4/4 (min-mod)
	f	0/4	1/4 (min)	3/5 (min-sli)	1/4 (mod)
Interstitial fibrosis	**m**	0/4	0/4	0/4	2/4 (sli-mod)
	f	0/4	0/4	0/5	1/4 (min)
Nodulular hyperplasia	**m**	0/4	0/4	0/4	1/4 (mod)
	f	0/4	0/4	0/5	0/4
GALLBLADDER					
Adhesive mucus	**m**	0/4	0/4	1/4 (min)	3/4 (sli-mod)
	f	0/4	0/4	2/5 (min-sli)	3/4 (min-sli)
Pseudogland formation	**m**	0/4	0/4	0/4	2/4 (sli-mod)
	f	0/4	0/4	0/5	2/4 (min-sli)
Increased lymphoid tissue	**m**	0/4	0/4	0/4	3/4 (min-sli)
	f	0/4	0/4	1/5 (sli)	3/4 (min-sli)

Data was not subjected to statistical analysis due to the limited number of animals per group

[1] *min = minimal; sli = slight; mod = moderate; mar = marked severity*

Relevant histopathological effects in the liver consisted of cytoplasmic change and hypertrophy, fatty change, and increased intrahepatocellular iron storage at 800 ppm and above. In the highest dose group, there were also focal necrosis and an increased number of binucleated hepatocytes. Two males and one female of the 8000 ppm group showed fibrosis of the liver parenchyma, in one animal this was accompanied by marked nodular hyperplasia. The fibrosis and hyperplasia were possibly a consequence of the high amount of stored iron. Adhesive mucus was observed in the gallbladder at 800 ppm and above and pseudogland formation was seen in the gallbladder wall in animals at 8000 ppm. Inactivity of the prostate was noted in 2 males from the highest dose group. In addition, one had severely reduced spermatogenesis of the testes and aspermia in the epididymes. However, this animal showed a marked reduction in general health in the form of significant emaciation and pronounced clinico-chemical and histopathological liver changes and these observations may be secondary.

The LOAEL is ≤ 80 ppm (2.6 mg/kg). At this dose there is evidence of adaptive change of the liver, but also some evidence possibly related to liver toxicity (slight increase in AP and ALAT and fatty change (1/4 males).

Study 7: <u>Supplementary 28 day feeding</u> study: liver enzyme induction. RAR B.6.3.1.3

In a GLP, non-guideline study, test substance (Iprovalicarb; 98.9% purity) was administered to 7 groups of 3 male and 3 female purebred (strain Bor. Beag) beagle dogs in the diet at concentrations of 0, 10, 20, 40 or 80 ppm over a period of 28 days, and 0 or 80 ppm in the two

recovery groups (28 days with test substance administration followed by a further 28 days recovery period without test substance exposure).

The calculated test compound intake in week 4 for males and females together was 0, 0.41, 0.77, 1.61, 3.01, and 2.93 mg/kg bw/day, corresponding to the dose groups of 0, 10, 20, 40, 80 and 80 ppm (recovery) respectively.

No deaths occurred and all animals appeared in good health through scheduled termination. There were no clinical observations which were considered test article-related. Body weight was unaffected. Food consumption was not reduced. Haematological parameters were not affected. At necropsy, no test-substance related gross pathological findingswere recorded. There were no treatment-related alterations in liver weights. There were no histopathological findings which could be attributed to test substance administration.

There was some microsomal liver enzyme induction in the 1.6 and 3 mg/kg bw dose groups (40 and 80 ppm respectively). There were increases in N-demethylase and O-demethylase activity and slight increases in cytochrome P-450. These effects (seen in the recovery group) were reversible.

Table 25: Microsomal liver enzyme induction (mean pooled values for males and females with statistical evaluation).

	N-DEM [mU/g]	O-DEM [mU/g]	P-450 [nmol/g]
Main groups	**m+f**	**m+f**	**m+f**
0 ppm	76.2	19.7	19.3
10 ppm	78.6	21.2	22.5
20 ppm	91.8	22.1	20.7
40 ppm	120.7++	24.7	23.5+
80 ppm	128.9++	26.2++	23.7+
Recovery groups	**m+f**	**m+f**	**m+f**
0 ppm	85.7	21.8	19.4
80 ppm	72.6	18.2	18.0
Laboratory reference value (1994-1995) - mean ± sd (range)	67.6±23(21.5-113.7) 69.5±17.54(34.4-104.6)	17.6±4.4 (8.8-26.4) 19.1±4.36(10.4-27.8)	18.5 ±4.25(10-27) 17.2±3.45(10-24.1)

U-test: + = 5% significance level; ++ = 1% significance level

4.7.1.2 Repeated dose toxicity: inhalation

Study 8: <u>5 day inhalation</u> study in Wistar rats: RAR B.6.3.4.1

Ten Wistar rats, strain Bor:WISW (SPF-Cpb) rats/sex were exposed to iprovalicarb dust (97.6 % purity) at concentration levels of 0, 20.6, 102.9 and 504.4 mg/m3 for a 6-hour period on each of 5 consecutive days, using “head/nose only” exposure. It was only possible to achieve the target respirability (particle mass ≤ 3µm) at concentrations up to about 500 mg/m3 air. Fifty percent of the animals were sacrificed on the 3rd follow-up day, and the other 50% were sacrificed at the end of the study. The exposure-free, follow-up observation period was 4

weeks. The MMAD was approx. 1.3 - 1.8 μm, the GSD approximately 1.9 - 2.5, the particle size < 3 μm and the aerodynamic diameter at least 80%.
There were no treatment-related mortalities, no clinical signs of toxicity and no effects on food consumption or body weights. There were no relevant alterations in haematological or clinical chemistry parameters. No concentration-level-related induction of the hepatic, mixed-function oxidases or effects on the triglyceride concentration was noted in the liver. At concentration levels up to 500 mg/m3, there was no relevant effect on organ weights, either absolute or relative. No evidence of any specific alterations to the organs was noted at either necropsy (interim or final). The LOEC was > 506 mg iprovalicarb/m^3, the maximum concentration achievable due to agglomeration of particles at higher concentrations.

4.7.1.3 Repeated dose toxicity: dermal

Study 9: 4 week subacute dermal toxicity study, HC:NZW rabbits. RAR B.6.3.4.2

The test substance was applied, occluded, to five New Zealand (HC:NZW) rabbits/sex for 6 hours per day for 22 days over a period of 4 weeks.

There were no treatment-related mortalities or clinical signs of toxicity during the study. There was no difference in feed consumption or body weights between the controls and the test animals. There were no signs of erythema during the study period, and, at no time during the study was the skin fold thickness of the treatment group greater than that of the control group. No treatment-related haematological or clinical chemistry effects occurred. There were no treatment-related pathological changes. There were no differences between the control and treatment groups with respect to either absolute or relative organ weights. No treatment-related histopathological changes were found in any of the organs or tissues examined.

Iprovalicarb was well tolerated following dermal exposure to 1000 mg/kg bw.

4.7.1.4 Repeated dose toxicity: other routes

No other data.

4.7.1.5 Human information

Not available.

4.7.1.6 Other relevant information

None available.

4.7.1.7 Summary and discussion of repeated dose toxicity

In rat, mouse and dog, the liver has been identified as the main target organ as indicated by higher liver weights associated with liver enzyme induction and/or hepatocellular hypertrophy in all 3 species. In addition, there was evidence of liver toxicity as marker enzymes were elevated in all three species. There was no histopathological collate in the rat and the mouse. The dog was the most sensitive species and the lowest short term LOAEL was established in female dogs following a 52-week treatment period on the basis of evidence of adaptive change and some evidence of toxicity (increased serum ALAT and AP, increased absolute and relative liver weight, liver enzyme induction from 2.6 mg/kg bw and increased serum enzyme activities,

increased liver weight, histopathology (cytoplasmic change, fatty change, intrahepatocellular iron storage) at 25 mg/kg.

Effects seen in the 52-week dog study at or near to the cut-off values for classification for repeated toxicity were not considered to be sufficient for classification under the criteria of the CLP Regulation (EC) No. 1272/2008.

4.8 Specific target organ toxicity (CLP Regulation) – repeated exposure (STOT RE)

4.8.1 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

The liver was the target of iprovalicarb in all three species tested. Clear adaptive change (increased relative and absolute liver weight was seen in the rat and mouse with altered clinical chemistry parameters indicative of toxicity also apparent in the rat at very high dose levels. The dog was the most sensitive, with adaptive change and also evidence of liver toxicity at high doses (≥ 2500 ppm/62.5 mg/kg) in the 90-day study (Study 5). Similarly, a clear adaptive response in the liver occurred in the 53-week study (Study 6) from 80 ppm/2.6 mg/kg. At this dose level there was some fatty periportal change in one male and evidence of iron in the hepatocytes in a single female.

4.8.2 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

STOT RE is assigned on the basis of findings of 'significant' or 'severe' toxicity. Significant findings are those which clearly indicate functional disturbance or morphological changes which are toxicologically relevant. Severe effects are more serious effects which impact on health. Effects seen in the 52-week dog study at or near to the cut-off values for classification for repeated toxicity were not considered to be sufficient for classification under the criteria of the CLP Regulation (EC) No. 1272/2008. While these effects are likely to represent the beginning of the treatment-related adverse effect, they are not considered to be significant/severe or likely to impact significantly on the animal's health.

4.8.3 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

Classification is not recommended.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal The DS analysed 9 different studies of different durations (from 28 days to 53 weeks), with different species (3 studies in rat, 1 study in mice, 4 studies in dog and 1 study in rabbit) and three different routes (7 studies oral, one dermal and one inhalation). DS detected the liver as the target organ in the three species, being dog the most sensitive. However, DS considered

the alterations in liver as adaptative changes and not causing significant impact on the animal's health. Therefore, DS proposed no classification of iprovalicarb for STOT RE.

Comments received during public consultation

Two different MSCAs disagreed with the DS's proposal and requested a discussion about a potential classification of iprovalicarb as STOT RE category 2 on the basis of the hepatotoxicity reported in dogs. DS acknowledged the proposal but disagreed and provided an extensive reply to the comments with information quite similar to the one already provided in the CLH dossier supporting their position.

Assessment and comparison with the classification criteria

Table 5 summarises the main findings in the repeated dose toxicity studies with iprovalicarb in animals.

Table 5: *Summary table for repeated dose toxicity studies in animals with iprovalicarb*

Method	Results	Reference
28 day feeding study OECD TG 407, GLP Wistar rat Males/females 10 animals/sex/group 99.4% iprovalicarb 0, 2000, 6000, 20000 ppm 0,0; 196/199; 579/573; 1974/1934 mg/kg bw/day Classification triggers with effects below 300 mg/kg bw/day	No dose-related effects at 2000 ppm **Effects of iprovalicarb on several end-points.** Shown as percentage regarding of control. * and ** = statistically different from control for p < 0.05 and 0.01, respectively.	Bomann, 1995 RAR B.6.3.1.1

	6000 ppm		20000 ppm	
	♂	♀	♂	♀
Alkaline phosphatase	115	113	132*	123
Cholesterol	103	122*	121**	137**
Triglyceride	107	159*	104	183*
Creatinine	82*	100	75**	98
Urea	68**	117	81**	91
O-demethylase	138**	155**	208**	216**
P-450 cytochrome	119	130*	137**	134**
Absolute liver weight	100	108*	105	117**
Relative liver weight	103	113**	111**	124**
Absolute pituitary weight	71**	71*	71**	86
Relative pituitary weight	67**	75	67*	100
Absolute thymus weight	90	100	87*	102
Absolute thyroid weight	120	133	120*	133
Relative thyroid weight	100	100	150*	150

Conclusions:
NOAEL = 2000 ppm (196/199 mg/kg bw/day)
LOAEL: 6000 ppm (579/573 mg/kg bw/day) for changes in liver weight and increase in P450 and O-demethylase activity > 300 mg/kg bw day → No classification

13-week feeding study with 4- week	No dose-related effects at 1250 ppm	Schladt *et al.*, 1996

recovery (high dose group)

OECD TG 408, GLP

Wistar rats

Males/females

10 animals/sex/group

98.1-98.7% iprovalicarb

0, 1250, 5000, 20000 ppm

0,0; 87/134; 373/561; 1524/2586 mg/kg bw/day

Classification triggers with effects below 100 mg/kg bw/day

Effects of iprovalicarb on several end-points. Shown as a percentage of the control values. * and ** = statistically different from control for $p < 0.05$ and 0.01, respectively. All effects were reverted at the end of the 4 weeks recovery period.

	5000 ppm		20000 ppm	
	♂	♀	♂	♀
Leucocytes	108	105	135**	103
Cholesterol	110	112	108	136**
Triglyceride	104	86	61**	85
N-demethylase	104	114	92	121*
O-demethylase	129**	133**	142**	190**
P-450 cytochrome	114	129**	134**	145**
Absolute liver weight	111	106	111	114**
Relative liver weight	109*	108*	117**	122**
Absolute heart weight	111	94	98	90**

Conclusions:
NOAEL: 1250 ppm (87/134 mg/kg bw/day)
LOAEL: 5000 ppm (373/561 mg/kg bw/day) for changes in liver weight and increase in P450 and O-demethylase activity > 100 mg/kg bw day → No classification

RAR B.6.3.2.1

2-years oral chronic toxicity

OECD TG 453, GLP

Wistar rats

Males/females

50 animals/sex/group

95.8-98.5% iprovalicarb in 1% peanut oil

0, 500, 5000, 20000 ppm

0,0; 26/32; 263/326; 1110/1380 mg/kg bw/day

Classification triggers

Vaginal bleeding in 4 females doses with 20000 ppm

Effects of iprovalicarb on several end-points. Shown as a percentage of the control values. * and ** = statistically different from control for $p < 0.05$ and 0.01, respectively.

	5000 ppm		20000 ppm	
	♂	♀	♂	♀
Amino aspartate transferase	77	48*	79	50*
Alanine amino transferase	82*	67	85	69
Alkaline phosphatase	116	114	143**	123
Glutamate dehydrogenase	29*	8*	18*	4*
Cholesterol	-	112	-	87
Total bilirubin	-	86	-	73*
Absolute liver weight	99	104	106	109*
Relative liver weight	99	109**	106	334*
Absolute kidney weight	100	97	94	91*
Relative kidney weight	104	102	95	100
Absolute spleen weight	88	89	89	82**
Relative spleen weight	86	91	88	89

Schladt and Hartmann, 1998

RAR B.6.5.1

with effects below 12.5 mg/kg bw/day

Histopathological findings.

		ppm			
		0	500	5000	20000
Hepatocellular hypertrophy	males	0/50	1/50	0/49	0/50
	females	1/50	2/50	8/48	20/50
Bile duct hyperplasia	males	20/50	24/50	29/49	31/50
	females	18/50	17/50	18/48	22/50
Focal Leydig cell hyperplasia		2/50	2/50	4/49	6/50

Conclusion:
NOAEL = 500 ppm (26.0-32 mg/kg bw/day)
LOAEL = 5000 ppm (263/326 mg/kg bw/day) for changes in liver morphology > 12.5 mg/kg bw day → No classification

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2-years oral chronic toxicity

OECD TG 451, GLP

B6C3F1 mice

Males/females

50 animals/sex/group

95.8-98.5% iprovalicarb in 1% peanut oil

0, 280, 1400, 7000 ppm

0,0; 59/97; 283/503; 1567/2544 mg/kg bw/day

Effects of iprovalicarb on several end-points. Shown as a percentage of the control values. * and ** = statistically different from control for $p < 0.05$ and 0.01, respectively.

	1400 ppm		7000 ppm	
	♂	♀	♂	♀
Amino aspartate transferase	140	56	107	58
Alanine amino transferase	198	50	97	47
Alkaline phosphatase	107	94	88	87
Glucose	75**	116*	82*	111
Triglyceride	102	131	87	114
Urea	126	114	113	114
Absolute liver weight	101	98	102	99
Relative liver weight	98	97	107	98
Absolute kidney weight	92**	100	83**	94
Relative kidney weight	89**	99	87**	93*

Histopathological findings.

		ppm			
		0	280	1400	7000
Liver fatty change	males	5/50	8/50	5/50	21/50
	females	2/50	1/50	4/50	7/50
Kidney tubular vacuolization	males	50/50	50/50	24/50	3/50
	females	0/50	0/50	0/50	0/50

Classification triggers with effects below 12.5 mg/kg bw/day

Conclusion:
NOAEL = 280 ppm (59 mg/kg bw/day)
LOAEL = 1400 ppm (283 mg/kg bw/day) for alterations in kidney weight > 12.5 mg/kg bw day → No classification

13 week feeding study

OECD TG 408, GLP

B6C3F1 mouse

Males/females

10 animals/sex/group (except 14000 ppm 6 animals/sex/group)

98.1-98.7% iprovalicarb

0, 280, 1400, 7000, 14000 ppm

0,0; 63/125; 325/697; 1725/3600; 3473/6869 mg/kg bw/day

Classification triggers with effects below 100 mg/kg bw/day

No dose-related effects at 280 and 1400 ppm

Effects of iprovalicarb on several end-points. Shown as a percentage of the control values. * and ** = statistically different from control for $p < 0.05$ and 0.01, respectively.

	7000 ppm		14000 ppm	
	♂	♀	♂	♀
RBC	98	99	96*	96
MCV	102	100	103	100
MCH	101**	102	103**	103
Cholesterol	110*	109*	98	111*
Absolute liver weight	107	106	118**	111
Relative liver weight	106	111**	117**	113**
Absolute kidney weight	94	101	90*	95
Relative kidney weight	93	106	89*	97

Conclusions:
NOAEL: 1400 ppm (325/697 mg/kg bw/day)
LOAEL: 7000 ppm (1725/3600 mg/kg bw/day) for increased liver weight and cholesterol level > 100 mg/kg bw/day → No classification

Watta-Gebert and Popp, 1996

RAR B.6.3.2.2

4 week feeding study

OECD TG 409, GLP

Beagle dogs

Males/females

2 animals/sex/group

98.1% iprovalicarb

0, 100, 1000, 10000, 50000 ppm

0,0; 3/3; 32/35; 280/270; 1322/1165 mg/kg bw/day

No dose-related effects at 100 ppm

Hepatocytes with ground-glass appearance (mild degree) and hypertrophy at 1000 ppm

Effects of iprovalicarb on several end-points. Shown as a percentage of the control values. * and ** = statistically different from control for $p < 0.05$ and 0.01, respectively. n = negative; m= Moderate; M = mild.

	10000 ppm		50000 ppm	
	♂	♀	♂	♀
Body weight gain		n	n	n
Activated partial thromboplastin time	90*	86	89*	85
Alkaline phosphatase	261	357**	636*	309**
GGT	221	177	386*	362
Glutamate dehydrogenase	168	67	816**	491
Cholesterol	47	109	55	59*
Urine pH	90	93	86*	79
Urine sodium	57	48*	46	25**

Porter *et al.*, 1993

RAR B.6.3.1.2

Classification triggers with effects below 300 mg/kg bw/day	Urine chloride	30*	26	32*	13*
	Urine potassium	28*	37	35*	23
	Absolute liver weight	105	129	105	123
	Relative liver weight	122	148	116	137
	Hepatocytes with hypertrophy	m	m	M	M
	Hepatocytes with ground-glass appearance	M	M	M	M

Conclusions:
NOAEL: 100 ppm (3/3 mg/kg bw/day)
LOAEL: 1000 ppm (32/35 mg/kg bw/day) for liver hypertrophy

13 week feeding study

OECD TG 409, GLP

Beagle dogs

Male/females

4 animals/sex/group

95.8-98.5% iprovalicarb

0, 250, 2500, 50000 ppm

0; 9.1; 62.5; 1250 mg/kg bw/day

No statistical analysis.

Data from sexes pooled

Vliegen and Hartmann, 1995

RAR B.6.3.2.3

Effects of iprovalicarb on several end-points. Shown as a percentage of the control value. No statistical analysis was provided. Data from males and females were provided pooled.

	ppm		
	250	2500	50000
Body weight	94	97	76
Alkaline phosphatase	113	180	395
Amino aspartate transferase	85	79	253
Alanine amino transferase	159	143	1634
Glutamate dehydrogenase	67	80	5133
Lactate dehydrogenase	82	86	144
Cholesterol	102	94	26
Protein	94	89	77
Albumin	90	83	58
Hepatic N-demethylase	258	346	256
Hepatic O-demethylase	194	251	164
P-450 cytochrome	212	322	345
Hepatic triglycerides	140	222	146
Absolute liver weight	113	133	131
Relative liver weight	119	139	169
Absolute testes weight	129	119	35
Relative testes weight	200	200	100
Absolute prostrate weight	91	78	35
Absolute thymus weight	105	95	43

Classification triggers with effects below 100 mg/kg bw/day

Liver histopathological findings.
m = minimal; s = slight; M = moderate.

		ppm			
		0	250	2500	50000
Cytoplasmic change	male	0/4	3/4	3/4	4/4
	female	0/4	2/4	4/4 S	3/4 M
Vacuolation	male	0/4	0/4	0/4	1/4
	female	0/4	0/4	0/4	2/4 M or s
Hypertrophy	male	0/4	0/4	0/4	2/4

	female	0/4	0/4	1/4 M	3/4 M
Multilamellar bodies	male	0/4	0/4	0/4	1/4
	female	0/4	0/4	1/4	2/4
Focal necrosis	male	0/4	0/4	0/4	3/4
	female	0/4	1/4 M	0/4	1/4 M or s
Single cell necrosis	male	0/4	0/4	0/4	4/4
	female	0/4	0/4	0/4	2/4 s or M
Iron pigments (Küpffer's cells)	male	0/4	0/4	0/4	2/4
	female	0/4	0/4	0/4	3/4
Iron pigments (periportal cells)	male	0/4	0/4	0/4	3/4
	female	0/4	0/4	0/4	1/4
Granulocytic infiltration	male	0/4	0/4	1/4	3/4
	female	0/4	0/4	0/4	1/4

Histopathological findings, organs other than liver.

		0 ppm	50000 ppm
Gallbladder: abnormal content	male	0/4	3/4
	female	0/4	2/4
Gallbladder: oedema wall	male	0/4	4/4
	female	1/4	3/3
Gallbladder: dilated lymphatic vessel	male	0/4	4/4
	female	0/4	2/3
Testes, juvenile		1/4	4/4 slight to massive
Epididymides, juvenile		1/4	4/4 moderate to massive
Prostrate, juvenile		1/4	4/4 marked to massive
Thymus, atrophy	male	0/4	4/4
	female	0/4	2/3
Bone marrow atrophy	male	0/4	3/4
	female	0/4	1/4

Conclusions:
LOAEL = 250 ppm (9.1 mg/kg bw/day) for histological findings in liver.

53 week feeding study	Reduction in bodyweight gain of males (but not in females) at 8000 ppm	Ruf and Rinke, 1997
OECD TG 452, GLP	**Effects of iprovalicarb on clinical chemistry at week 53.** It Shown as a percentage of the control value.	

Beagle dogs

Males/females

4 animals/sex/group

98.7% iprovalicarb

0, 80, 800, 8000 ppm

0,0; 2.6/2.7; 24.7/28; 257/262 mg/kg bw/day

Classification triggers with effects below 25 mg/kg bw/day

No statistical analysis

RAR B.6.3.3.1

	80 ppm		800 ppm		8000 ppm	
	♂	♀	♂	♀	♂	♀
Amino aspartate transferase	87	92	86	97	231	119
Alanine amino transferase	209	125	232	114	1623	523
Alkaline phosphatase	174	86	255	121	1771	329
Glutamate dehydrogenase	61	93	103	128	2903	1072
Albumin	93	96	97	85	68	78

Effects of iprovalicarb on microsomal liver induction at week 53. Shown as a percentage of the control value.

	80 ppm		800 ppm		8000 ppm	
	♂	♀	♂	♀	♂	♀
N-demethylase	201	171	416	443	437	474
O-demethylase	137	171	241	248	232	267
P-450 cytochrome	138	131	247	265	374	410
Triglycerides	103	121	133	213	142	180

Effects of iprovalicarb on organ weights at week 53. Shown as a percentage of the control value.

	80 ppm		800 ppm		8000 ppm	
	♂	♀	♂	♀	♂	♀
Absolute liver	110	109	125	111	134	141
Relative liver	115	103	123	115	160	131
Relative adrenal	112	-	133	-	152	-

Liver histopathological findings.
m = minimal; s = slight; M = moderate.

		ppm			
		0	80	800	8000
Cytoplasmic change	male	0/4	0/4	4/4 (m-s)	4/4 M
	female	0/4	0/4	4/5 s	4/4 M
Hypertrophy	male	0/4	0/4	4/4 m	4/4 s-M
	female	0/4	0/4	4/5 m-s	4/4 s-M
Multilamellar bodies	male	0/4	0/4	0/4	1/4 m
	female	0/4	0/4	0/5	3/4 s-M
Periportal fatty change	male	0/4	1/4 s	3/4 m-s	2/4 M
	female	0/4	0/4	3/5 m-s	1/4 m
Binucleated	male	0/4	0/4	0/4	3/4 m-s
	female	0/4	0/4	0/5	2/4 m-s
Focal necrosis	male	0/4	0/4	0/4	2/4 m-s
	female	0/4	0/4	0/5	0/4

Single cell necrosis	male female	1/4 m 1/4 m	0/4 0/4	0/4 0/5	1/4 m 4/4 m
Iron pigments	male female	1/4 m 0/4	0/4 1/4 m	0/4 3/5 m-s	4/4 m-M 1/4 M
Interstitial fibrosis	male female	0/4 0/4	0/4 0/4	0/4 0/5	2/4 s-M 1/4 m
Nodular hyperplasia	male female	0/4 0/4	0/4 0/4	0/4 0/5	1/4 M 0/4

Gall bladder histopathological findings.
m = minimal; s = slight; M = moderate.

		ppm			
		0	80	800	8000
Adhesive mucus	male female	0/4 0/4	0/4 0/4	1/4 m 2/5 m-s	3/4 s-M 3/4 m-s
Pseudo gland formation	male female	0/4 0/4	0/4 0/4	0/4 0/5	2/4 s-M 2/4 m-s
Increased lymphoid tissue	male female	0/4 0/4	0/4 0/4	0/4 1/5 s	3/4 m-s 3/4 m-s

Conclusions:
NOAEL = 80 ppm (2.6/2.7 mg/kg bw)
LOAEL = 800 ppm (24.7-28 mg/kg bw) for liver effects (slight increase in liver transferases in clinical chemistry and alterations in fatty change).

Supplementary 28 day feeding study: liver enzyme induction

GLP

Beagle dogs

Males/females

3 animals/sex/group

98.9% iprovalicarb

0, 10, 20, 40, 80 ppm (additional

Microsomal liver enzyme induction. Shown as a percentage of the control value. * and ** = statistically different from control for p< 0.05 and 0.01, respectively.

	N-demethylase	O-demethylase	P-450 cytochrome
0 ppm	100	100	100
10 ppm	103	108	117
20 ppm	120	112	107
40 ppm	158**	125	122*
80 ppm	169**	133**	123*
Recovery groups			
0 ppm	100	100	100
80 ppm	85	83	93
Reference values	89 97	89 97	96 89

Conclusions:

Wetzig and Rinke, 1997

RAR B.6.3.1.3

groups for 0 and 80 ppm for assessing recovery) 0, 0.4, 0.8, 1.6, 3 mg/kg bw/day	**NOEL for enzyme induction: 20 ppm (0.8 mg/kg bw/day)** **LOEL for enzyme induction: 40 ppm (1.6 mg/kg bw/day)** **for increases in N-demethylase and O-demethylase P-450 cytochrome.**	
5 day inhalation study Wistar rats OECD TG 412, GLP 97.6% iprovalicarb 0, 21, 103, 504 mg/m3 High dose = max. technically feasible concentration MMAD: approx. 1.3-1.8 µm Particle size at least 80% <3 µm	504 mg/m^3 No effects **Conclusions:** **NOAEC = 504 mg/m3**	Pauluhn, 1993 RAR B.6.3.4.1
4 week subacute dermal toxicity OECD TG 410, GLP HC:NZW rabbits 95.8% iprovalicarb 0, 1000 mg/kg bw/day Limit dose	1000 mg/kg bw/day No effects **Conclusions:** **NOAEC = 1000 mg/kg bw/day**	Vohr and Geiß, 1995 RAR B.6.3.4.2

Data presented in the table 5 clearly show that liver is the target organ of repeated toxicity induced by iprovalicarb and that dog is the most sensitive species. However, the moderate to severe hepatotoxicity found in oral rat (28 days, 13 weeks and 2 years) and mouse (13 weeks and 2 years) studies was reported at doses far above the guidance values for classification as STOT RE category 2. One inhalation study in rat and one dermal study in rabbit showed no toxicity at doses above the reference values therefore were not suitable for classification purposes.

The hepatotoxicity reported in several studies in dog showed increases in absolute and relative liver weight, alterations in clinical chemistry and urinalysis compatible with liver impairments

and also histopathological impairments as cytoplasmic change, vacuolation, hypertrophy, multilamellar bodies, focal and single cell necrosis, iron pigments in Küpffer's and periportal cells and granulocytic infiltration. The table 6 summarises the hepatotoxicity reported in dog studies at doses below the reference values for warranting classification:

Table 6: *Summary of hepatotoxicity induced by iprovalicarb found the oral repeated dose toxicity studies in dog.*
Table shows only those adverse effects displayed in the table 5 appearing at dose levels below the cut-off points considered in guideline for warranting classification.

Study	Effect	Dose (mg/kg bw/day)	STOT RE Cat. 2 (mg/kg bw/day)
4 weeks	Hepatocytes with ground-glass appearance (mild and moderate degree) and hypertrophy.	32/35 and 280/270	Lower than 300
13 weeks	Histopathological findings: cytoplasmic change (7/8), hypertrophy (1/8), multilamellar bodies (1/8), granulocytic infiltration (1/8). Microsomal liver induction. Minimal effects in clinical chemistry (no statistical assessment provided).	62.5	Lower than 100
53 weeks	Histopathological findings: cytoplasmic change (8/9); hypertrophy (8/9); periportal fatty change (6/9); iron pigments (3/9). Microsomal liver induction. Minimal effects in clinical chemistry (no statistical assessment provided). Gall bladder histopathological findings: adhesive mucus (3/9) and increased lymphoid tissue (1/5).	24.7 (males) 28 (females)	Lower than 25
Supplementary 28 day	Microsomal liver induction.	1.6	Lower than 300

RAC notes that the dose-selection in the 13-weeks study in dogs (9.1; 62.5 and 1250 mg/kg bw/day) prevents a conclusive assessment of the relevance of the effects in testes, prostrate and epididymis as well as on thymus and bone marrow. Nevertheless, RAC also notes that no information on reproductive or immune system effects is reported in the 53 week study; which suggests that the effects seen in the 13 week study are not reproducible and therefore of less concern in relation to classification.

The classification for STOT RE is appropriate when significant or severe toxicity in target organs is observed. RAC notes that very severe hepatotoxicity was found in several studies in dogs at doses several times higher than the classification guidance values (table 5); while the effects in liver at doses below the guideline reference values (table 6) likely represents the beginning of the treatment-related adverse effects reported at higher doses and they are not considered to be significant/severe or likely to impact significantly on the animal's health for warranting classification.

In conclusion, RAC supports the DS's proposal for **no classification of iprovalicarb as regards as STOT RE.**

4.9 Germ cell mutagenicity (Mutagenicity)

Table 26: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
Point mutation			
Salmonella microsome assay (Ames test)* *S. typhimurium* (TA 98, TA 100, TA 1535, TA 153 AS: 98.1 % pure 8 - 5000 µg/plate (± S9)	Negative		Herbold, B.A., 1994 RAR B.6.4.1.1
Salmonella microsome test: S. typhimurium (TA102) AS: 96.8% pure 16 - 5000 µg/plate	Negative		Herbold, 2001; 31331 B.6.4.1.2
HPRT assay: Chinese hamster lung (V79) cells AS: 98.1 % 7.8 - 250 µg/ml (+S9) 12.5 - 125 µg/ml (-S9)	Negative		Brendler-Schwaab, S., 1995 RAR B.6.4.1.4
Salmonella typhimurium and E. coli reverse mutation assay *S.typhimurium :* TA98, TA100, TA1535, TA1537, *E.coli* : WP2/uvrA 3 - 5000 µg/plate Iprovalicarb 95.7%	Negative		Sokolowski, 2012; 1466200 RAR B.6.4.1.3
Chromasomal aberration test			
Cytogenetic assay: Chinese hamster ovary (CHO) cells AS: 98.7 % pure. 6, 30, 150 µg/ml	Negative		Gahlmann, R., 1995 RAR B.6.4.1.5
Cytogenetic study- 18 hours treatment (-S9) Chinese hamster V79 cells AS: 96.86% 0,120,180 mg/ml	Negative		Herbold, 2001; 31333 RAR B6.4.1.6
DNA damage			
Unscheduled DNA synthesis (UDS) test: Primary rat hepatocytes AS: 98.1 - 99.4 % pure. 50 - 500 µg/ml	Negative		Brendler-Schwaab, S., 1996 RAR B.6.4.1.7
Mammalian micronucleus test			
Micronucleus test (MNT) NMRI mouse bone marrow cells AS: 96.7 % pure. 2000 mg/kg bw (i.p.)	Negative		Herbold, B.A., 1995 RAR B.6.4.2.1

DNA damage			
32P-Postlabelling assay: *uterus and urinary bladder of rats AS: 96.4 % pure. 20000 and 10000 ppm in the feed	Negative		Brendler-Schwaab, S., 1998 RAR B.6.4.2.2

*to investigate DNA adduct formation in these organs

4.9.1 Non-human information

4.9.1.1 *In vitro* data

Iprovalicarb was negative in all *in vitro* test systems evaluated.

4.9.1.2 *In vivo* data

Iprovalicarb was negative in all *in vivo* test systems evaluated.

4.9.2 Human information

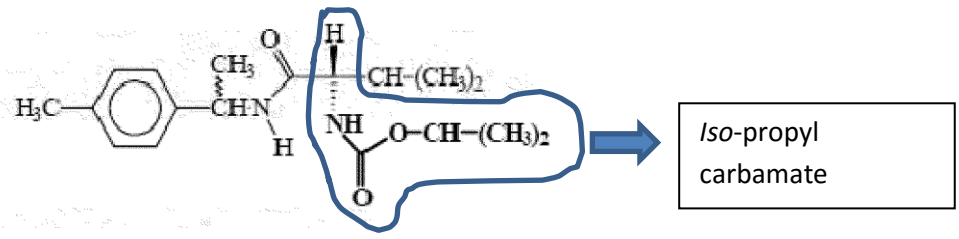
No human data.

4.9.3 Other relevant information

DEREK QSAR analysis of iprovaricarb (see 4.10.3).

Jeffery, A. M., NY Medical College, Department of Pathology, Chemical Saferty Laboratory, Valhall, NY, USA (Report No 28122011; December 2011): Iprovalicarb: Evaluation of Deductive Estimation of Risk from Existing Knowledge (DEREK) based in Structure Activity Relationships (SAR).

A QSAR analysis of iprovalicarb identified no alerts for carcinogenicity and one alert for genotoxicity, evaluated as 'plausable' rather than 'probable'. This was based on the presence of an alkyl carbamate moiety in the molecule which is clearly related to vinyl carbamate which generates a DNA-reactive epoxide.



Summary

The structural alert was considered by the notifier not to be relevant for iprovalicarb for the following reasons:

1 . Vinyl carbamate is genotoxic owing to its metabolism to oxiram-2-yl carbmate (vinyl carbamate epoxide), while *iso*-propyl carbamate is considered difficult to metabolically activate towards a reactive epoxide. There is no evidence in the ADME studies presented for

the formation of such a metabolite from iprovalicarb itself or release of *iso*-propyl carbamate from iprovalicarb.

2. The *p*-methylphenyl group of iprovalicarb is readily oxidised to carboxylic acid and conjugated. This represents the major and non-genotoxic pathway for iprovalicarb and substancially reduces the possibility of metabolism at the *iso*-propyl carbamate moiety, and hence potential carcinogenicity.

Neither metabolism study identified any metabolite derived from oxidation of the valine *iso*-propyl carbamate group, which would be the pathway for metabolic activation to a genotoxic metabolite based on the DEREK alert. However, it is recognised as a theoretical possibility. It is acknowledged that identification of such metabolites would be difficult owing to their instability.

There is no specific experimental basis to support the *in silico* assessment of the structural alert by vinyl carbamate: that the *iso*-propyl carbamate moiety, (for which there is no evidence for equivalent metabolic activation to a reactive epoxide in the case of Iprovalicarb), may pose a genotoxic/carcinogenic risk.

4.9.4 Summary and discussion of mutagenicity

Iprovalicarb was sufficiently tested for all relevant endpoints and was negative.

4.9.5 Comparison with criteria

Classification for mutagenicity or genotoxicity is made on the basis of evidence from reliable and acceptable *in vitro* and *in vivo* tests or studies in somatic or germ cells of exposed animals. OECD Test Guidelines compliant studies were submitted and evaluated. Since there is no evidence of mutagenic activity or increased incidences of heritable mutations for iprovalicarb there is no classification proposed.

4.9.6 Conclusions on classification and labelling

Classification is not required.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal DS proposed no classification of iprovalicarb for germ cell mutagenicity on the bases of a wide array of *in vivo* and *in vitro* test all with negative results. **Comments received during public consultation** One MSCA supported the DS's proposal for no classification of iprovalicarb as regards as germ cell mutagenicity.

Assessment and comparison with the classification criteria

Tables 7 and 8 resume the *in vitro* and *in vivo* genotoxicity studies with iprovalicarb.

Table 7: *Summary table of relevant in vitro mutagenicity studies with iprovalicab*.

Method	Test system	Tested concentrations	Results	Remarks	Reference
Ames test OECD TG 471, GLP	*S. typhimurium* (TA 98, TA 100, TA 1535, TA 153)	8- 5000 µg/plate of 98.1% iprovalicarb	With S9: negative Without S9: negative	At 4000 µg/ plate, the substance started to precipitate Positive controls worked well with and without S9 mix	Herbold, 1994 RAR B.6.4.1.1
Ames test OECD TG 471, GLP	*S. typhimurium* (TA102)	16-5000 µg/plate of 96.8% iprovalicarb	With S9: negative Without S9: negative	At 5000 µg/ plate, the substance started to precipitate Positive controls worked well with and without S9 mix	Herbold, 2001; 31331 B.6.4.1.2
HPRT assay OECD TG 476, GLP	Chinese hamster lung (V79) cells	7.8- 250 µg/ml (with S9) 12.5-125 µg/ml (without S9) 98.1% iprovalicarb	With S9: negative Without S9: negative	Positive controls worked well with and without S9 mix	Brendler-Schwaab, 1995 RAR B.6.4.1.4
Ames test OECD TG 471, GLP	*S. typhimurium* (TA98, TA100, TA1535, TA1537) E.coli (WP2/uvrA)	3-5000 µg/plate of 95.7% iprovalicarb	With S9: negative Without S9: negative	Positive controls worked well with and without S9 mix	Sokolowski, 2012; 1466200 RAR B.6.4.1.3
Cytogenetic assay OECD TG 473, GLP	Chinese hamster ovary (CHO) cells	6, 30, 150 µg/ml of 98.7% iprovalicarb	With S9: negative Without S9: negative	Positive controls worked well with and without S9 mix	Gahlmann, 1995 RAR B.6.4.1.5
Cytogenetic study - 18 hours treatment (without S9)	Chinese hamster V79 cells	0,120,180 mg/ml of 96.86% iprovalicab	With S9: negative	Positive controls worked well with and without S9 mix	Herbold, 2001; 31333

OECD TG 473, GLP			Without S9: negative		RAR B6.4.1.6
Unscheduled DNA synthesis (UDS) test OECD TG 482, GLP	Primary rat hepatocytes	50-500 µg/ml of 98.1-99.4% iprovalicab	With S9: negative Without S9: negative	The test material was excessively toxic at a concentration of 300 µg/ml Dose levels at or below 150 µg/ml were non-toxic. Positive controls worked well with and without S9 mix	Brendler-Schwaab, 1996 RAR B.6.4.1.7

Table 8: *Summary table of relevant in vivo mutagenicity studies with iprovalicab.*

Method	Test system	Tested concentrations	Results	Remarks	Reference
Micronucleus test (MNT) OECD TG 474, GLP	NMRI mouse bone marrow cells	2000 mg/kg bw (i.p.) of 96.7% iprovalicab	Negative	Systemic toxicity: apathy, roughened fur, spasm, difficulty in breathing, diarrhoea	Herbold, 1995 RAR B.6.4.2.1
^{32}P-postlabelling assay	Uterus and urinary bladder of rats	20000 and 10000 ppm in the feed of 96.4% iprovalicab	Negative		Brendler-Schwaab, 1998 RAR B.6.4.2.2

No positive results were found in a wide battery of well-performed and reliable tests both *in vitro* and *in vivo*. Thus, RAC supports the DS's proposal for **no classification of iprovalicarb for germ cell mutagenicity.**

4.10 Carcinogenicity

Table 27: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Combined chronic toxicity/	≥5000 ppm (females):		Schladt, L., and Hartmann, E.,

carcinogenicity study, Wistar rat Iprovalicarb: 95.8 - 98.5% pure 0, 500, 5000, 20000 (26/31.7, 262/326, 1109.6/1379.7)	decreased body weights, clinical chemistry, higher relative liver weights and increased incidence of hepatocellular hypertrophy. ↑mixed Muellerian tumours and thyroid tumours 20000 ppm (males): ↓body weight and increased AP activity. ↑malignant skeletal tumours. 20000 ppm (females): ↑papillomas of the urinary bladder		1998 RAR B.6.5.1
Oncogenicity study, B6C3F1 Iprovalicarb: 95.8 - 98.5% pure 0, 280, 1400, 7000 (58/97, 283/503, 1566.8/2544/0 280 (58.5)	≥1400 ppm (both sexes): slightly ↑ blood urea levels, slightly ↓ kidney wt (males). 7000 ppm (males): marginally ↓ bw, slight ↑ food and water intake, ↑ triglyceride serum levels and degenerative changes in liver cells with associated marginally ↑ rel. liver wt.		Leser, K.H. and Vogel, O. 1997 RAR B.6.5.2
Proliferating Cell Nuclear Antigen Iprovalicarb: 98.8%-98.5% 0, 500, 5000, 20000 (26/31.7, 262/326, 1109.6/1379.7) .	↑RF values in some bone/urothelial cells/uterus sections of questionable biological relevance		Latropoulus, M.J., Williams, M.J. 2006 RAR B.6.5.3

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Study 1: Chronic toxicity and carcinogenicity investigations in Wistar rats. RAR/RAR B.6.5.1

Materials and Methods:

Groups of 50 male and 50 female Wistar (Hsd/WIN:WU) rats each were administered iprovalicarb (95.8 - 98.5% purity) *ad libitum* in their diet at concentrations of 0, 500, 5000, or 20000 ppm for 24 months. Ten additional rats/sex and dose were included in the study for an interim sacrifice after 12 months of treatment. The study conformed to the requirements of OECD Guideline 453 with no deviations. In addition, blood samples for the determination of hormones were taken at the end of the study as well as liver specimens during interim and final necropsy for determination of Phase I and Phase II enzyme activities.

Test diet: 1% peanut oil was added to all mixtures to minimize the generation of dust. The average test substance intake corresponding to the doses of 0, 500, 5000, and 20000 ppm, respectively, was 26.0, 262.5, and 1109.6 mg/kg bw (males) and 31.7, 326.3, and 1379.7 mg/kg bw (females).

Findings

Mortality/clinical signs: There was no evidence of a substance-related effect on mortality. Vaginal bleeding occurred in a higher incidence in the 20000 ppm females (3, 4, 2, 10). No other observations or clinical signs of toxicity. There were no treatment-related ophthalmological effects.

Body weights/food consumption: Body weights of males were marginally retarded in the 20000 ppm group (maximum reduction was 8%). As the study progressed (from week 61), the body weight values were almost identical to controls. In the 20000 ppm females, the reduction in bodyweights compared to controls was statistically significant at most time points (maximum reduction was 12%). From week 12 onwards, statistically significant differences frequently occurred in 5000 ppm females also (maximum reduction 7%); however, at study termination, the reduction of body weights was marginal compared to controls (5% decreases, statistically non-significant). Water intake was normal.

Haematology: Red blood cell parameters showed no evidence of a treatment-related effect in males and females up to and including 20000 ppm. One high dose female had vaginal bleeding and a uterine tumour, which resulted in a tendency to lower (not statistically significant), values for erythrocyte counts, haemoglobin concentration, haematocrit values and consequent increases in proportion of reticulocytes in females in the 20000 ppm group.

Clinical chemistry: AP was significantly increased in the high dose males from week 53 onwards. No treatment-related effect was seen in females. Cholesterol was increased (significantly in weeks 27 and 53) in high dose females. Total bilirubin concentration was slightly but significantly decreased in high dose females in weeks 27, 79 and 105. Electrolyte concentration in peripheral blood gave no indication of treatment-related effects. The results of urinalysis revealed no evidence of treatment-related effects.

Table 28: Summary of clinical chemistry

		0 ppm		500 ppm		5000 ppm		20000 ppm	
Week		**53**	**105**	**53**	**105**	**53**	**105**	**53**	**105**
AST [U/l]	m	41.4	31.2	38.9	26.9	30.0	24.0	25.7+	24.6
	f	61.6	61.8	59.9	52.4	31.9+	29.4+	26.7+	30.6+
ALT [U/l]	m	44.5	32.2	34.7	28.6	33.4	26.4+	29.8++	27.4
	f	52.7	50.1	62.9	42.4	40.5	33.4	39.0	34.8
AP [U/l]	m	181	164	178	176	214	191	224+	235++
	f	137	117	144	106	125	133	145	144
GLDH [U/l]	m	24.1	16.9	13.4	12.5	4.1	4.9+	0.5+	3.0+
	f	51.3	62.3	50.4	45.0	5.8+	6.2+	2.4+	2.5+
CHOL [nmol/l]	f	2.45	2.88	2.76	3.69	2.99	3.23	3.42++	3.18
BILI-t [mcmol/l]	f	2.0	2.2	1.9	2.0	1.9	1.9	1.9	1.6+

Dunnett test: + = 5 % significance level ++ = 1 % significance level

Organ weights: The mean relative liver weight of high dose females was increased (22%) at interim sacrifice. Relative weights of the brain and kidneys were also significantly increased in this group. At terminal sacrifice, an increase of mean relative liver weight at 5000 and 20000 ppm (9% and 19%, respectively) and absolute liver weight at 2000 ppm (9%) was noted in females.

Table 29: Summary of organ weights

		0 ppm	500 ppm	5000 ppm	20000 ppm
interim sacrifice (12 months)					
Abs. liver wt. [mg]	**m**	19262	18071	20144	20925

	f	10778	10305	10648	11548
Rel. liver wt. [mg/ 100g bw]	**m**	3526	3293	3563	3243
	f	3422	3142	3637	4189**
Abs. kidney wt. [mg]	**m**	3400	3402	3407	3335
	f	2041	2011	1959	2060
Rel. kidney wt. [mg/100 g bw]	**m**	622	620	602	612
	f	649	616	670	750**
terminal sacrifice					
Abs. liver wt. [mg]	**m**	18486	18653	18332	19637
	f	11759	12295	12204	12782*
Rel. liver wt. [mg/100 g bw]	**m**	3481	3477	3438	3695
	f	3612	3700	3954**	12782*
Abs. kidney wt. [mg]	**m**	3590	3698	3596	3388
	f	2382	2519	2307	2160*
Rel. kidney wt. [mg/100 g bw]	**m**	679	691	703	642
	f	731	765	749	729
Abs. spleen wt. [mg]	**m**	1214	1286	1067	1082
	f	776	762	687	633**
Rel. spleen wt. [mg/100 g bw]	**m**	229	241	198	202
	f	238	231	216	213

Dunnett test: * = 5 % significance level **= 1 % significance level

Histopathology

Non-neoplastic change: Some female rats in the 5000 ppm (2 rats) and 2000 ppm (3 rats) showed slightly enlarged hepatocytes in the interim sacrifice group. Other histopathological findings observed were considered to be of spontaneous origin.

Hepatocellular hypertrophy was seen in the livers of females from ≥ 5000 ppm (1/2/8/20, respectively) in animals scheduled for 24-months treatment. This adaptive response correlated with increased liver weights and is indicative of an increased metabolic activity.

A slight increase in the incidence, but not the severity, of focal bile duct hyperplasia was observed in males from ≥ 5000 ppm. In high dose females, an increased haematopoiesis was observed in the femoral and sternal bone marrow. The incidences of diffuse hyperplasia in the mammary gland appeared somewhat lower in 20,000 ppm females. Diffuse and focal C-cell hyperplasia in the thyroid gland occurred less frequently in the 20,000 ppm females, whereas similar incidences of focal hyperplasia of the follicular epithelium were observed in all groups of both sexes.

A slight increase in focal hyperplasia of the *pars distalis* of the pituitary gland was seen increased in all male treatment groups and in 20000 ppm females, but fell well within the historical control range.

The incidence of focal Leydig cell hyperplasia was slightly increased in testes of 20000 ppm males. A pair-wise group comparison was not performed. However, a positive trend (<0.05) was established.

Table 30: Non-neoplastic histopathological findings

	Males				Females			
Dose (ppm)	**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
Liver - no. animals examined	50	50	49	50	50	50	48	50
Hepatocellular hypertrophy	0	1	0	0	1 t**	2	8**	20**
Bild duct hyperplasia	20 t**	24	29	31	18	17	18	22
Testes - no. animals examined	50	50	49	50				
Leydig cell hyperplasia, focal	2 t*	2	4	6 $				

t* = p<0.05, t** = p<0.01 (trend-test according to Peto et al., 1980)
* = p<0.05 (one-tailed pairwise group comparison according to Peto et al., 1980)

Neoplastic changes:

A C-cell adenoma was noted in the thyroid of one control female (killed at interim sacrifice). A sarcoma of the skin occurred in one female in the 500 ppm group (killed in moribund state in week 50). A malignant solid basalioma of the skin was diagnosed in one 5000 ppm male (killed in moribund state in week 40), and another male of this dose group had a malignant mixed glioma in the cerebrum (killed in moribund state in week 47). All of these findings are considered to be of spontaneous origin.

The distribution and breakdown of neoplasms found in animals scheduled for the 2-year treatment can be seen in Table 23. There appeared to be a shift in tumour incidences at the high dose level of 20000 ppm.

Table 31: Neoplastic change in animals at terminal sacrifice.

Incidence of tumors: average incidence% (range%) Animals scheduled for terminal sacrifice									
Sex		**Male**				**Female**			
Dose (ppm)		**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
Urinary bladder No. exam.		50	50	49	50	50	49	48	50
Papilloma, Transitional Cell	b	0	0	0	0	0 t*	0	0	2(4%) ns
Bayer Pharma /HC data *-All 23 studies: 1986-2007* *-12 studies in 5 year range/ 1993-2003* *RITA data base HC (119 studies: 1984-2009)*		*0.00.0* *0.7% (0.0-15.0)*				*0.1% (0.0-2.0)* *0.0* *2.2% (0.0-2.2)*			
Mammary gland No. exam.		50	50	49	49	50	49	48	50
Fibroadenoma	b	0	0	0	1	8	6	11	3
Adenoma	b	0	0	0	0	0	1	0	0
Adenocarcinoma	m	0	0	0	0	6 t**	3	2	0 $
Uterus No. exam.						50	50	48	50
Adenocarcinoma	m					2	3	3	6 ns

Incidence of tumors: average incidence% (range%) Animals scheduled for terminal sacrifice									
Sex		**Male**				**Female**			
Dose (ppm)		**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
Mixed Muellerian Tumor (MMT)	m					0 t*	0	1	2 (4%) ns
(MMT) *Bayer Pharma /HC data* *All (22 studies: 1986-2007* *-13 studies in 5 year range: 1993-2003* *RITA data base HC (110 studies)*		- 				 0.1% (0.0-1.7) 0.1% (0.0-1.7) 0.1%(0.0-2.0)			
Pituitary gland No. exam.		50	50	49	50	50	50	48	50
Adenoma / Pars Distalis	b	11	6	10	12	20	19	20	14
Thyroid gland No. exam.		50	50	49	50	50	50	48	50
Adenoma / Follicular Cell	b	1	1	2	0	0t*	0	1	2 (4%)ns
Carcinoma / Follicular Cell	m	0	0	0	0	0	0	1	1 (2%)
†Bayer Pharma/HC data (HED CARC iprovalicarb 2002 -adenoma -carcinoma *RITA data base HC (119 studies)* -adenoma -carcinoma		- 				 (0.6%) 0.0-0.2 (0.0) 00.0-0.0 0.0-6.1% 0.0-5.0%			
Skeletal system No examined		50	50	49	50	50	50	48	50
Osteosarcoma									
-Femur/knee joint	m	0 t*	0	0	2 ns	0	0	0	0
-Bone, other site[#]					1				
-total		0	0	0	3(6%)	0	0	0	0
Chondrosarcoma (Nasal cavity)[#]	m	0	0	0	1	0	0	0	0
HC Bone (NOS) *-Bayer Pharma /HC data (20 studies)* *-RITA data base HC (108 studies)*		 0.1% (0.0-1.7) 0.2%(0.0-2.0)				 0.0 0.1%(0.0-4.0)			
Clitoral gland [#] no exam..		-	-	-	-	50	50	48	50
Carcinoma / Squamous Cell	m					0	0	0	2(4%)
Bayer Pharma /HC data (2 studies) *RITA data base HC (21 studies)*		- 				2.3-3.0% 0.5%(0.0-4.3)			

= organ / tissue not routinely examined; t* = p<0.05, t** = p<0,01 (trend-test according to Peto et al., 1980); * = p<0.05 (one-tailed pairwise group comparison according to Peto et al., 1980); $ = no pairwise group comparison performed; ns = not significant; b = benign; m = = malignant.

†Thyroid tumour historical data not from relevant time interval..

Skeletal system: Three males of the 20000 ppm group, which died or had to be killed in a moribund state, were diagnosed with malignant tumours of the skeletal system; 2 metastasising osteosarcomas of the femur, 1 osteosarcoma of the lower jaw, and 1 chondrosarcoma of the nasal cavity. Chondrosarcoma and osteosarcoma are considered to have a common etiology but are not considered together as the nasal cavity is not normally examined. The incidence of osteosarcoma of the bone observed in male rats (6%) exceeded the highest historical control incidence rate in both the updated in-house laboratory database (average 0.1% (range 0.0 – 1.7%) in males and 0.0% in females) and the RITA historical control database for males (0.2% (0.0 – 2%)) and females (0.1% (0.0 -4.0%)). There were no control data available for chondrosarcomas.

Malignant mixed Muellerian tumours: Malignant mixed Muellerian tumours in the uterus were observed in one of the 5000 ppm female and in two 20000 ppm females. In one high dose animal, metastases of this tumour type had occurred in several organs. Trend test analysis was statistically significant (p = 0.038) for this lesion while a dose adjusted pairwise group comparison did not achieve statistical significance. These tumours are rare in rats and are postulated to originate from pluripotent mesodermal cells of the mullerian duct. These tumours are characterised by an admixture of malignant epithelial and mesenchymal components. In the heterologous type seen in this study, the epithelial structures consist of glandular, squamous, or anaplastic epithelium, while the mesenchymal parts differentiate towards osteosarcoma and/or chondrosarcoma. The incidence of this tumour exceeded the highest historical control incidence rate in the updated in-house laboratory historical control data-base (0%) and the RITA database (2%). Mixed muellerian tumours occurred in 6 cases/6004 animals (0.1%) in the complete RITA data base (1984-2009) and in 3/3585 (0.08%) in studies conducted within 5 years of this study. Spontaneous frequencies per study vary between 0 and 2% (RITA data set). A malignant mixed muellerian tumour was recorded in a low dose group of a separate current 2 year study with Wistar rats (BASF T 9059203). A relationship to treatment cannot be excluded on the basis of the information provided. Adenocarcinoma incidence was slightly higher in the 20000 ppm group than in the other treatment groups, but the difference was not significant.

Transitional cell papilloma: Transitional cell papillomas (benign) were found in the urinary bladder of two 20000 ppm females (4%) (not statistically significant in a trend test analysis ($p<0.031$)). There were no neoplastic findings in the bladder of males. Increased incidences of hyperplastic/pre-neoplastic lesions of the transitional epithelium such as focal or diffuse hyperplasia were not observed in either sex. There were no transitional cell carcinomas observed in the female rats and no transitional cell adenomas or carcinomas in the male rats in this study. Transitional cell papillomas in females are recorded in the RITA data base with a spontaneous frequency of between 0-2.2%. The study incidence for this rare urinary bladder tumour therefore exceeded the relevant historical background incidence for both this laboratory (0-2.0%) and the extended RITA data base. A relationship to treatment cannot be excluded on the basis of the information provided.

Thyroid follicular cell tumours: An increase in the incidence of follicular cell adenoma was observed in females at 5,000 (not significant) and 20,000 ppm (4%). This exceeded the average of the in-house laboratory historical control database evaluated by the reviewer*(average 0.6%) with a range of 0-2%. The highest historical control data for the extended RITA (Bayer AG) data base was 6.1%, (see Reference 2 below). There was a significant positive trend in the incidence of adenomas (p = 0.03; 0, 0, 1, 2).

An increased incidence in follicular cell carcinoma (2%) was observed in thyroids of 20,000 ppm females which exceeded the highest historical control rate of the in-house database (0%) but did not exceed the highest historical control incidence rate (5%) registered in the RITA data base. Pre-neoplastic lesions (focal hyperplasia of the follicular epithelium) were seen in similar incidences in all groups. Other lesions indicating an effect on the thyroid gland such as hypertrophy were not reported for iprovalicarb. Follicular cell adenomas and carcinomas of the thyroid are infrequently observed in Wistar rats and relationship to treatment is considered on the basis of the information provided. The incidence of thyroid follicular cell adenoma, adenocarcinoma and adenoma/carcinoma combined in female rats in the iprovalicarb rat study is therefore outside the relevant HC incidence.

*Historical control data referred-to by the industry for this tumour were not considered relevant as the reference related to studies conducted between 1975 and 1980[1]. For this reason, other data were assessed to address this tumour incidence.

1. **Bomhard, E., and Rinke, M.** Frequency of spontaneous tumours in Wistar rats in 2-year studies. Exp. Toxic. Pathol. 1994; 46: 17-29.

This was the HC data referred to by the study author in the rat 2-year study (B.6.5.1). This publication describes the historical data in 22 2-year studies using Wistar (TNO/W74- now called WISW (SPF Cpb)). These studies were initiated from May 1975 to December 1980 in Bayer AG Wuppertal, Germany and therefore do not fall within 5 years of the study in question (1998 ± 5 years = 2003-1993). .

Thyroid tumour incidences:

Follicular adenoma:	Follicular carcinoma:
♂average 1.1% -range 0.0-4.4	♂average 0.5% -range 0.0-2.0%
♀ average 0.9% - range 0.0-6.5	♀ average 0.2% - range 0.0-2.1

Additional HC data were assessed by the reviewer and are described below.

2. **Eiben, R. and Bomhard, E.m**. Trends in mortality, body weights and tumour incidences of Wistar rats (Bor:WISW (SPF Cpb – bred Winkelmann, Borchen, Germany) over 20 years. Exp Toxic Pathol 1999; 51: 523-536.

   This publication reviews the trends in important parameters over 20 years in Wistar rat long term studies carried out in the Bayer AG Institute of Toxicology, Wuppertal (1975-1994) and includes the data referred to in reference 1. The possible shift in the background incidence of tumours was assessed. With regard to thyroid tumours, it was concluded that benign or malignant follicular cell tumours were observed relatively infrequently (mean 1.4% in males (18.5% of which were malignant) and 0.8% in females (8.3% of which were malignant)). In most studies the frequency was between 0 and 2%. Higher frequencies (3-6%) were seen in a very few groups between 1975-1991 in males and between 1975 and 1980 in females, but not thereafter. It was therefore concluded that there was a somewhat decreasing trend in the later studies but without statistical significance. On the basis of this information, the incidence of thyroid adenoma and carcinoma in the present study appears higher than HC data for both adenoma and carcinoma.

3. **HED 0050652. Cancer Assessment Document:** Evaluation of carcinogenic potential of iprovalicarb, April 2002. US EPA CARC HED OPP.

   Historical data from BAYER AG Wuppertal were presented in this report which have not been submitted (for thyroid tumours) during the AIR II review. These data are from fourteen 2-year studies completed between 1991 and 1998 using Wistar (BOR:WISW (SPF Cpb) strains Hsd/WIN:WU and Hsd Cpb:WU.

Follicular adenoma:	Follicular carcinoma:
♂average 0.7% -range 0.0-4.0	♂average 0.1% -range 0.0-2.0%
♀ average 0.6% - range 0.0-2%	♀ average 0.0% - range 0.0-0.0

Some other historical control data are published on Wistar rats used in carcinogenicity studies in other locations.

4. **Carlus, M., *et al.*** Historical control data of neoplastic lesions in the Wistar Hannover Rat (RjHan:WI – bred in Janvier, France) among eight 2-year carcinogenicity studies. Experimental and Toxicological Pathology 65 (2013) 243-253.

   In this study, the data are gathered from 8 successive 2-year carcinogenicity studies at the Bayer CropScience Research Center (France) from 2001-2013.

Follicular adenoma:	Follicular carcinoma:
♂average 0.4% -range 0.0-2.0	♂average 0.6% -range 0.0-1.7%
♀ average 0.2% - range 0.0-1.7%	♀ average 0.4% - range 0.0-2.0

5. **Poteracki J. and Walsh, K.** Spontaneous neoplasms in control Wistar Rats: A comparison reviews. Toxicological Sciences 45; 1-8 (1998).

   In this study, the data are generated from 5 carcinogenicity studies conducted over a 5 year period (1990-1995). The rats were Wistar (Crl:(WI) BR) from Charles River, NY or MI.

Follicular adenoma:	Follicular carcinoma:
♂average 3.9% -range 1.7-6.9	♂average 0.9% -range 0.0-1.7%
♀ average 2.8% - range 2.0-3.3%	♀ average 1.5% - range 0.0-3.3

Overall, the indication is that the range quoted by the applicant for this study is not appropriate or relevant and that additional data (Eiben and Bomhard, 1999 and the US HED CARC report for iprovalicarb) gives a better indication of the real background in

this tumour in female Wistar rats in this facility (BASF AG Wuppertal) in the appropriate time frame. Eiben and Bomhard,(1999) clearly identifies that the high values for adenoma (in females) in the RITA data base relate to studies conducted prior to 1980 and are not relevant to the present study. The data presented in the US EPA HED report are the most relevant and give an average incidence in female rats of 0.6% with a range of 0 – 2% for follicular cell adenoma and 0% with a range of 0 for follicular cell carcinoma. The incidences of thyroid follicular cell adenoma, adenocarcinoma and adenoma/carcinoma combined in female rats in the iprovalicarb rat study are therefore outside the relevant HC incidence.

Clitoral gland: Two females of the 20000 ppm group had a squamous cell carcinoma of the clitoral gland (p = 0.089). Clitoral glands are not protocol organs and not normally examined in carcinogenicity studies therefore lesions not seen at necropsy will not be detected. Spontaneous clitoral gland tumours are infrequent in rats. The incidence rate in two other studies where this gland was examined in the test laboratory was 2.4 – 3.0% (2 studies) and in the extended RITA database was 0.5% (range of 0.0 – 4.3%). The biological significance of this finding cannot be assessed in the absence of histological examination of this gland in all other high dose animals in the study.

The other tumour types which occurred were not increased in frequency in any treatment group and they are known from previous studies with rats of this strain.

The numbers of tumour bearer animals, among the animals dying intercurrently or killed in moribund conditions which were originally scheduled for 24-month treatment, and among the animals killed at terminal sacrifice are shown in Table 23. The somewhat increased number of metastasising tumours at 20,000 ppm among the females dying intercurrently results from the prevalence in the increased number of metastasising uterine tumours. Considering all animals, however, there is no dose-related increase in either sex in the frequency of tumour bearers, the number of animals with exclusively benign or exclusively malignant, or with benign and malignant tumours.

Table 32 Tumour-bearing animals dying intercurrently or killed at termination

Animals with tumours Animals scheduled for terminal sacrifice									
Sex		**Male**				**Female**			
Dose (ppm)		**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
Intercurrently died or killed animals									
Number examined		19	20	14	12	17	20	14	17
Animals with:	tumours (total)	7	15	10	9	15	14	12	14
	benign only	5	7	4	4	10	8	9	7
	malignant only	2	7	4	4	4	3	1	6
	malignant and benign	0	1	2	1	1	3	2	1
	metastasising	1	2	1	2	1	2	0	5
Terminal kill									
Number examined		31	31	35	38	33	30	34	33
Animals with:	tumours (total)	21	17	16	25	26	19	24	21
	benign only	17	13	15	24	17	12	17	15
	malignant only	1	1	0	1	5	1	2	2
	malignant and benign	3	3	1	0	4	6	5	4
	metastasising	0	0	0	0	2	3	1	1
All animals									
Number examined		50	50	49	50	50	50	48	50
Animals with:	tumours (total)	28	32	26	34	41	33	36	35
	benign only	22	20	19	28	27	20	26	22

Animals with tumours Animals scheduled for terminal sacrifice								
Sex	**Male**				**Female**			
Dose (ppm)	**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
malignant only	3	8	4	5	9	4	3	8
malignant and benign	3	4	3	1	5	9	7	5
metastasising	1	2	1	2	3	5	1	6

A survey of the occurrence of animals with tumours over time at intervals of 13 weeks is shown in Table 24. The results only consider those animals which died intercurrently, but were originally scheduled for 24 month treatment. There is no evidence of any effect of treatment on the premature occurrence of tumours. It can be seen that there was no dose-related increase in the frequency of the total number of tumours occurring or in the incidence of benign and malignant neoplasms alone, in either sex.

Table 33. Occurrence of tumours over time.

Sex		**Male**				**Female**			
Dose (ppm)		**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
No. of animals intercurrently died or killed		19	20	15	12	17	20	16	17
Tumours[§:]	benign	6	10	7	7	16	11	19	10
	malignant	2	9	6	5	5	7	3	7
	total	8	19	13	12	21	18	22	17
No. of animals with tumours:									
Week	1 - 13	-	-	-	-	-	-	-	-
	14 - 26	-	-	-	-	-	-	-	-
	27 - 39	-	-	1	-	-	-	-	-
	40 - 52	-	-	1	-	-	1	-	1
	53 - 65	1	2	-	2	-	3	1	2
	66 - 78	-	-	1	-	-	1	3	-
	79 - 91	4	4	3	1	8	3	1	4
	92 - 104	2	8	5	6	5	6	6	6
	105 - 107		1			2	1	1	1

Conclusion and discussion:

Although the high dose level was in excess of 1000 mg/kg bw/day, there was no serious systemic toxicity based on the observed decreases in body weight gain and food efficiency, increased liver weight and hypertrophy in females, occurrence of bile duct hyperplasia and Leydig cell hyperplasia in males. There was no effect on survival. The effects on body weights in males at 20,000 ppm and females at 5,000 ppm (difference to controls: maximally 8% and 7% respectively) were relatively small and not significant at most time points, and can be considered to be borderline effects. Feed and water intake were unaffected.

Changes in liver morphology and function, beginning at 5,000 ppm, were predominantly noted in females. They can be considered to be an adaptive response due to an inducing effect on microsomal enzymes with corresponding slight effects on lipid metabolism. The incidence of focal bile duct hyperplasia was slightly increased in males, but as this is a common finding in aging male rats and was not severe in any group, it is most likely to be of spontaneous origin. No biliary neoplasms were observed. The dosing was considered to be adequate for the

assessment of chronic toxicity and carcinogenicity even though the high dose was in excess of 1000 mg/kg bw/day.

Malignant tumours of the skeletal system were seen in 20,000 ppm males. There were 3 incidences of osteosarcomas (two of the femur and one of the lower jaw). Historical data showed incidences of spontaneously occurring osteosarcomas in the range of 0 - 2%. In addition, one chondrosarcoma of the nasal cavity was diagnosed. Historical data on chondrosarcoma are not available. Spontaneous osteosarcoma is uncommon in both laboratory animals and man and particularly rare in rats. Osteosarcoma can be induced by ionising radiation, alkylating agents, and has been shown to be induced at a high frequency by long term administration of human PTH (1-34) and (1084) to Fisher rats presumable by a skeletal anabolic effect *via* an unknown mechanism (Tashjian, A.H., and Goltzman, D., J. of Bone and Mineral Research. Volume 23; (6) 803-811 (2008). The RF values in all treated groups were slightly and significantly increased at 12 months and could not be measured in the 24 month sections. While there was no evidence of a preneoplastic change in bone eg., hyperostosis, a relationship to treatment cannot be disregarded.

There was an increase in malignant mixed Muellerian tumours in the uteri of females treated at 5000 and 20000 ppm. Spontaneous incidences per study documented in the RITA data base varied between 0% and 2%. In a supplementary ^{32}P-postlabelling assay performed with uterine tissue after treatment of rats with iprovalicarb gave no evidence of DNA-adduct forming potential of the test substance (RAR B.6.8.3.2.4). A PCNA study indicated a statistically significant increase in proliferation in all treated tissues when compared to controls (x 1.48, x1.55 and x1.93 fold increase at the low, mid and high doses respectively). This is not considered biologically relevant by the study author as they state that PCNA value increases only start to be meaningful at approximately 3-fold compared to controls (see RAR B.6.5.3). The significance of this finding is difficult to assess and the information considered supplementary.

The incidence of uterine adenocarcinomas was slightly increased at 20000 ppm (12%). However, there was no significant trend shown here, and was within the historical control data ranges given.

In two females at 20,000 ppm, squamous cell carcinoma of the clitoral gland was observed. Clitoral glands are not routinely investigated, but only when some macroscopic changes are visible. Therefore, all animals in the high dose and controls were not examined and the biological significance of this finding is difficult to assess. The incidence (2%) at the high dose was within the updated historical control incidence of 2.3-3.0% and relationship to treatment is not considered to be likely.

Benign neoplastic lesions of the urinary bladder (transitional cell papilloma) were observed in two females at 20000 ppm. No pre-neoplastic lesions of the urothelium were observed. The incidence in this study (4%) is above the range of historical values, which could indicate a treatment-related effect. A supplementary ^{32}P-postlabelling assay performed with urinary bladder epithelium after treatment of rats with iprovalicarb gave no evidence of DNA-adduct forming potential of the test substance (RAR B.6.8.3.2.4). However, the PCNA study data (increased RF above control $p<0.05$) infer that there is an increased proliferation in this tissue at 24 months which may be driving the tissue towards a slight increase in papilloma at this high dose (see B.6.5.3 below for PCNA data) and supported a possible relationship to treatment.

There was a statistically significant positive trend in the incidence of thyroid follicular cell adenomas (5,000 and 20,000 ppm groups) and an increase in carcinoma and

adenoma/carcinoma combined which exceeded the relevant historical control data. However, other lesions indicating an effect on the thyroid gland such as hypertrophy have not been reported in this study or in other toxicity studies with the test substance, and pre-neoplastic lesions in the present study were seen in similar incidences in all groups. No effect was seen in the thyroids of males.

In conclusion, the histopathological findings indicate a shift in the incidences of certain tumours at 5,000 ppm (mixed Muellerian uterine tumours, thyroid follicular cell adenomas), and at 20,000 ppm (transitional cell papillomas of the urinary bladder in females, and skeletal system tumours in males). None of these tumour types was seen in control animals, and are only rarely observed in long-term studies. Although the incidences of these tumours were low, they are all either extremely rare or uncommon in Wistar rats. Data from a ^{32}P-postlabelling assay performed with uterine tissue and urinary bladder epithelium *in vivo* gave no indication of DNA-adduct formation. A PCNA assay on neoplastic and non-neoplastic tissues from tumour-bearing tissues in the rat study indicated some increases in RF values in non-tumour tissues, possibly consistent with increased proliferation in these tissues. These increases were considered by the authors to be non-biologically relevant. The relevance of these findings to the assessment of the carcinogenicity of iprovalicarb was difficult to assess.

Therefore, although one mechanistic data study was provided which suggest that iprovalicarb is not a tumour initiator, no mode of action could be established for induction of any tumour type and a possible relationship to treatment could not be excluded.

It should be noted that the rats were subjected to significantly high doses (1379.7 mg/kg bw for females and 1109.6 mg/kg bw for males), as the test substance was observed to be of very low general toxicity in subchronic studies and there was no evidence of genotoxicity in the adequate data submitted.

Study 2: Oncogenicity study in B6C3F1-Mice

Iprovalicarb (95.8 - 98.5%) was administered via the food to groups of 50 male and 50 female Wistar (B6C3F1) mice at concentrations of 0, 280, 1,400, or 7,000 ppm over a period of up to 105 weeks. Ten additional mice/sex/dose were treated likewise and were sacrificed after 52 weeks. To minimize the generation of dust, 10 g peanut oil/kg food was added to all mixtures. The study was conducted in accordance with OECD 451 without significant deviations.

Findings:

Mortality/Clinical signs: There was no evidence of any substance-related effect on mortality. No abnormalities were noted with respect to body surfaces and orifices, general behaviour, posture, respiration and excretory products in any of the treated animals.

Body weights/food consumption: Body weights of males were marginally lower in the 7,000 ppm group only (from the beginning to the end of the study by about 4.1%) in comparison with controls. Body weight development was unaffected in all treated females. The food intake values of males and females in all dose groups did not differ significantly. Averaged over the study period, the following quantities of iprovalicarb were taken up daily with the diet, corresponding to the doses of 0, 280, 1,400, and 7,000 ppm, respectively: 58.5, 283.4, and 1566.8 mg/kg bw (males) and 97.4, 503.1, and 2544.0 mg/kg bw (females).

Haematology: Haematological examinations did not show treatment-related effects on haematological parameters.

Clinical chemistry: Mean plasma activities of AST and ALT, AP and GLDH were comparable with control values in all treatment groups. In week 104, the values for these enzymes were extremely high in a few male rats (2 in the 280 ppm group and 2 in the 1400 ppm group), which were correlated with liver tumours observed in these animals. Slight shifts in AP activity and glucose concentration were noted in certain animals, but given that the differences were small and dose-dependence was lacking, they were not considered to be of any toxicological significance. A tendency to slightly higher values were observed in the urea concentrations of males at 1,400 ppm and above at weeks 53 and 104, and in females at 1,400 ppm and above at week 104. Triglyceride concentrations were significantly higher in males at 7,000 ppm in week 53, but slightly lower than in controls at the end of the treatment period (week 104).

Table 34: Clinical chemistry

		0 ppm		280 ppm		1400 ppm		7000 ppm	
Week		**53**	**104**	**53**	**104**	**53**	**104**	**53**	**104**
AST [U/l]	m	29.1	30.5	29.2	61.6	27.9	42.8	24.9	32.6
	f	30.8	53.0	28.8	29.3	27.9	29.9	35.8	31.0
ALT [U/l]	m	24.9	24.4	22.5	54.8	34.4	48.4	20.6	23.6
	f	22.3	44.8	23.1	20.7	19.5	22.5	24.3	20.9
AP [U/l]	m	93	121	88	190	93	130	81++	106
	f	179	509	152	459	165	480	155	441
GLUC [mmol/l]	m	5.54	4.99	5.38	4.08+	5.44	3.75++	5.59	4.08+
	f	5.74	5.25	4.99	6.16+	5.69	6.09+	6.59	5.83
TRIG [mmol/l]	m	2.07	2.82	2.69	2.50	2.80	2.87	3.50++	2.44
	f	*	0.86	*	1.06	*	1.13	*	0.98
UREA [mmol/l]	m	9.97	12.69	10.91	13.29	11.08	15.98	12.03++	14.31
	f	9.87	12.36	10.48	13.10	9.77	14.05	9.81	14.04

Dunnett test: + = 5 % significance level ++ = 1 % significance level
* Sample volume insufficient in most animals to allow meaningful evaluation

Organ weights: At interim sacrifice, male mice at 1,400 ppm and above had lower absolute ($p \leq 0.05$ at 7,000 ppm) and relative ($p \leq 0.01$ at both dose levels) kidney weights. Absolute ($p \geq 0.05$) and relative ($p \leq 0.01$) liver weights were higher for males at 7,000 ppm. Other organ weights did not differ markedly from controls in either sex. At terminal sacrifice, absolute and relative kidney weights were significantly lower in males at 1,400 ppm and above. Females at 7,000 ppm had lower absolute (not significant) and relative ($p \leq 0.05$) kidney weights. Females at 1,400 ppm and above had significantly lower absolute and relative spleen weights. Males were unaffected. Absolute and relative liver weights were higher in males at 280 ppm due to the higher incidence of hepatocellular neoplasms. Absolute (males and females) and relative (females only) heart weights were in the 7,000 ppm groups. No other changes in absolute or relative organ weights of the animals treated with iprovalicarb were biologically significant.

Table 35: Organ weights

Necropsy (week)		**53**				**105**			
Dose level (ppm)		**0**	**280**	**1400**	**7000**	**0**	**280**	**1400**	**7000**
Kidney									
Abs. wt. (mg)	**m**	672	705	603	582+	738	750	679++	613++
	f	396	411	411	403	481	497	481	451

Rel. wt. (mg/100 g bw)	**m**	1777	1816	1555++	1565++	2022	2060	1803++	1762++
	f	1389	1406	1387	1417	1642	1654	1621	1529+
Liver									
Abs. wt. (mg)	**m**	1719	1844	1823	2019	1923	2210+	1942	1960
	f	1358	1407	1418	1411	1603	1578	1573	1584
Rel. wt. (mg/100 g bw)	**m**	4565	4737	4659	5364++	5242	6048+	5137	5620
	f	4748	4807	4772	4963	5467	5276	5287	5382

Dunnett test: + = 5 % significance level, ++ = 1 % significance level

Histopathology:

Non-neoplastic changes: Non-neoplastic alterations in the liver and the kidneys, both of which appeared to be treatment-related, were noted in animals scheduled for two years of treatment, including those which died spontaneously or were sacrificed.

Significant increases in the incidence of fatty change (clear-cut intracellular vacuoles), in single or groups of liver cells distributed in the parenchyma, were recorded in the livers of high dose male mice. The lesions present evidence of degenerative hepatocellular changes. In the kidneys, the incidence of tubular vacuolization was markedly decreased from 1400ppm in males only.

Table 36: Non-neoplastic histological findings

Main Groups (all animals)								
Dose (ppm)	**0**	**280**	**1400**	**7000**	**0**	**280**	**1400**	**7000**
Sex	**m**	**m**	**m**	**m**	**f**	**f**	**f**	**f**
No. of animals	**50**	**50**	**50**	**50**	**50**	**50**	**50**	**50**
Liver								
-"Fatty change"	5	8	5	21	2	1	4	7
Kidney								
-Tubular vacuolization	50	50	24	3	0	0	0	0

Neoplastic changes: There was no evidence that the tumours (benign and/or malignant) which were recorded throughout the study (including mice which died intercurrently) were treatment-related.

A higher incidence of hepatocellular neoplasms was recorded in males at 280 ppm in comparison with controls or other treated males (incidence of hepatocellular adenoma plus carcinoma in ascending order of dose: 28% - 50% - 26% - 28%), indicating no dose relationship. No remarkable increase in the incidence of hepatocellular neoplasms was observed in the females up to and including 7,000 ppm. As the B6C3F1 mouse strain is known for its relatively high incidences of hepatocellular neoplasms ranging from 7% to 58% in control males, and the incidence of hepatocellular neoplasms does not show a dose relationship, the increased number of liver tumours in males at 280 ppm cannot be linked to treatment with iprovalicarb. There was no evidence of any other increase of tumour incidences associated with treatment.

Table 37: Liver tumour incidences

		0 ppm	280 ppm	1400 ppm	7000 ppm
No. of examined animals		50	50	50	50
Liver					
adenoma (b)	m	7 (14%)	15 (30%)	7 (14%)	7 (14%)
	f	1 (2%)	1 (2%)	2 (4%)	4 (8%)
carcinoma (m)	m	7 (14%)	10 (20%)	6 (12%)	7 (14%)
	f	2 (4%)	1 (2%)	1 (2%)	0 (0%)

Conclusion and discussion:

Mortality was unaffected by treatment with iprovalicarb. Average food intake per animal was unaffected by treatment, but in relation to body weights, the mean food intake/kg body weight in males at 7,000 ppm was slightly higher than in animals of the other dose groups. As observed in the subchronic study in mice, mean water intake per kg body weight was slightly higher in males at 7,000 ppm than in controls. In all other treatment groups, water intake was comparable to controls. Body weights in males at 7,000 ppm were marginally reduced compared to controls. Otherwise, there were no clinical effects. Haematology parameters were unaffected by treatment.

Slightly higher triglyceride concentration in males at 7,000 ppm in week 53 was not apparent at the end of the study. Histopathological examination showed a significant increase in the incidence of fatty change in the hepatocellular parenchyma in male mice at 7,000 ppm. Absolute and relative liver weights were also somewhat increased in males at 7,000 ppm, statistically significant for the relative weight at week 53 only.

Clinical chemistry also revealed a slightly higher blood urea concentration in males (weeks 53 and 104) and in females (week 104) at 1,400 ppm and above, indicating a possible minor impairment of kidney function. This finding is corroborated in males at 7,000 ppm by the higher water intake. In males at 1,400 ppm and above, absolute and relative kidney weights were lower. However, the overall weight of evidence does imply a possible effect on kidney function beginning at 1,400 ppm.

Gross and histopathological investigations into other organs and tissues gave no indication of test-compound related changes in either sex up to or including 7,000 ppm.

A higher incidence of hepatocellular neoplasms was recorded in males in the 280 ppm group was considered unrelated to treatment. Other neoplastic lesions observed were not treatment-related and were those commonly diagnosed in mice of this age and strain.

In summary, daily administration of iprovalicarb admixed in the feed at dose rates of up to 280 ppm in males, and 7,000 ppm in females for a period of 2 years did not produce any adverse effects, and similarly doses of up to 7,000 ppm did not produce any evidence of a carcinogenic effect of the test article in either sex. There is no evidence of any tumourigenic potential associated with the administration of iprovalicarb to mice over a two year period.

4.10.1.2 Carcinogenicity: Inhalation

No data.

4.10.1.3 Carcinogenicity: dermal

No data.

4.10.2 Human information

No data.

4.10.3 Other relevant information

Study 3: Proliferating cell nuclear antigen (PCNA) immunohistochemical evaluation report on selected target tissues from SZX072 (Iprovalicarb): Chronic toxicity and carcinogenicity investigations in Wistar rats (administration in the feed over 24 months.

Introduction.

Iprovalicarb was administered in the feed to groups of 50 Wistar (Hsd/WIN:WU) rats/sex/dose level for 24 months at dose levels of 0, 500, 5,000, or 20,000 ppm (equivalent to 0, 26, 263, or 1,110 mg/kg/day in males and 0, 32, 326, or 1,380 mg/kg/day in females, reported above (section B.6.5.1). An increased incidence of rare and uncommon tumours was recorded (see above). In order to monitor the rate of proliferation in these possible target tissues, proliferating cell nuclear antigen (PCNA) immunohistochemical stain was performed on bone and cartilage tissues in males and urinary bladder (UB), and uterus and thyroid tissues in females from archival material (blocks) from the 24 month terminal sacrifices from 8 randomly selected samples per group of rats, including the samples with neoplasms. Subsequently, an additional 10 rats from the 12 month interim segment of the study were evaluated. The bone tissues from this group were decalcificed with ethyldiaminetetraacetic (EDTA) to improve the staining.

PCNA evaluation

Paraffin sections of the urinary bladder, uterus, thyroid gland, bone, cartilage and tumour samples about five microns thick were cut and de-paraffinised. For gentle heat pre-treatment, the sections were placed in a citrate buffer bath at a temperature of 90ºC and put in an incubator for 60 minutes. For enhancement of the immunoreaction in bone tissue, one additional set of bone slides was pre-treated in a pressure cooker for 3 minutes.

All sections were then incubated with anti PCNA (clone PC 10, Biogenex), dilution 1:80 for 30 minutes at room temperature. Subsequent steps were performed according to the avidin-biotin-method (Vectastain ABC kit Mouse Peroxidase IgG, Camon). The immunoreaction was visualised by addition of 3,3 diamino-benzidine tetrachloride (DAB) and the enzyme substrate hydrogen peroxide. Finally, the sections were counterstained with haematoxylin. The slides were then shipped to New York Medical College, Department of Pathology, in Valhalla, NY, for evaluation.

For quantitation of bone/cartilage, urinary bladder, uterine and thyroid gland cells, a square graticule (Olympus Instruments, Inc.) with 25 equal subdivisions was used at 400× magnification. Each side of the graticule was confirmed by measurement with a second micrometer (Graticules, Ltd., Tonbridge, UK) as 0.125 mm, providing a square counting area of 0.0156 mm2 that usually contains 5-20 bone/cartilage cells, 10-70 thyroid follicular cells,

20-50 uterine cells, and 10-80 urothelial cells. Five of the most PCNA positive areas were enumerated for each rat. In addition, areas from inside the neoplasm in the target tissues (urinary bladder (UB), uterus, thyroid) were counted. Again, the most proliferative areas were selected for counting. Based on the above, the labelling indices of each tissue were calculated, reflecting the percent replicating fraction (RF).

Response criteria (communication from the author (Gary Williams , NY Medical College, US, March 2013): Benchmark uterine PCNA value (%) increases were given for the oestrogenic chemicals tamoxifen and DES, of ~3 to ~5-fold increases compared to controls. Based on 15 years' experience of this laboratory, PCNA value increases are considered to be meaningful at ~ 3-fold and greater compared to controls. For statistical comparisons of each exposed group to controls, the Student-t-test was used.

Results:

Bone: Male bone, bone marrow and cartilage tissue samples from adult Wistar rats were evaluated in order to establish the order of magnitude of response in the various types of cells found in osseous tissue, see Table 27. The values in all three iprovalicarb treated groups at 12 months were slightly but significantly ($p<0.05$) elevated by pair-wise comparison to controls (flat dose response and no significant trend). In male 24 month bone and cartilage tissue samples, the percent RF group means could not be obtained because these tissues were PCNA negative despite the presence of PCNA-positive elements within blood vessels, e.g. white blood cells, and also in the bone marrow (Table 28). The percent RF within 2 osteosarcomas was 28 in No. 203 (tumour in the lower jaw, which was lethal to the rat on week 53) and 38 in No. 220 (tumour in the femur/knee joint, with lung metastases, which was lethal on week 94). The third osteosarcoma was PCNA negative (No. 187, of the femur/knee joint with lung metastases, lethal on week 89). Likewise, the single osteochondroma was PCNA negative (No. 199, of the nasal cavity, which was lethal on week 101, Table 28.

Table 38: Rate of proliferation in osseous tissue and bone marrow (BM) from the proximal femur of adult Wistar rats.

Cell Identification And Parameter	Total Osseous and BM Tissue Cells	Hematopoietic BM Cells	Para-Trabecular Osteoblasts	Stromal BM Cells	Para-Trabecular Osteoclasts	Trabecular Osteocytes
Total cells counted	3170 [a]	2818 [b]	208 [c]	102 [c]	32 [d]	10 [e]
Percent of total cells	100	90	6.6	3.2	1.0	0.32
Total PCNA positive cells	703	551	106	54	2	<1
Percent of PCNA positive cells	100	78	15.1	7.7	0.3	<0.1

RF, percent replicating fraction derived from proliferating cell nuclear antigen (PCNA)
positive nuclei over total nuclei counted from 10 graticule areas with the highest PCNA stain intensity. The age of rats consisted of a combined value derived from the 12 month and the 24 month sacrifices.

a composite of haemotopoitic (≈90%), osteoblasts (≈6.6%), stromal (≈3.2%), osteoclasts (≈1%) and osteocytes (≈0.3%).
b mean value with a range of ≈1595-3105.

c mean value with a range of ≈93-354.
d mean value with a range of ≈19-42.
e mean value with a range of ≈5-15.

Table 39: Comparison in male rats of benign and malignant neoplasms and the rate of proliferation in bone

Group, Dose (mg/kg/d)& Number (N)	Neoplasms: Rat Number	Neoplasms: Time of Observation (weeks)	RF within Neoplasm	RF Outside Neoplasm	Group Mean RF	Group Range RF
	12 Month Interim Sacrifice (the bones were decalcified with EDTA)					
	Bone [a]					
1. 0 (9)	NA	NA	NA	NA	19	15-24
2. 26 (9)	NA	NA	NA	NA	24*	21-31
3. 263 (10)	NA	NA	NA	NA	24*	18-30
4. 1110 (8)	NA	NA	NA	NA	24*	16-32
	24 Month Terminal Sacrifice (TS) (the bones were decalcified with formic acid)					
1. 0 (8)	NA	NA	NA	NA	NE	NE
2. 26 (0)	NA	NA	NA	NA	TNE	TNE
3. 263 (0)	NA	NA	NA	NA	TNE	TNE
4. 1110 (12)	187**	89, MA	NE	NA	33 ***	28-38
	203**	53, MA	NE	28	33 ***	28-38
	220**	94, MA	NE	38	33 ***	28-38

RF, percent replicating fraction derived from PCNA positive nuclei over total nuclei counted, taken from areas of highest PCNA positive incidence; [a], a composite of hematopoietic (~60%), stromal (~30%) and osseous (~10%) cells in adult rats, the osseous cells consist of osteocytes (~4%), located within the trabeculae, osteoblasts (~3%), and osteoclasts (~3%) both located on the surface of the trabeculae; NA, not applicable; NE, tissue could not be evaluated; TNE, tissue was not examined; MA, malignant neoplasm; *, significant at $p<0.05$ in a pair-wise comparison to group 1, but not biologically significant; **, significant at $p<0.05$ in a pair-wise comparison to group 1, and showing a significant trend at $p<0.01$; ***, the mean value was obtained from the two RF values (28 and 38) from outside the neoplasms.

Uterus: Uterine (luminal/stromal/myometrial) mean RF values in controls at 12 months were 38 (range 26-48) and at 24 months were 29 (range 19-35). This fall in RF is considered to reflect the normal gradual atrophy with age. At 12 months, the RF values in the low and mid dose groups were similar to controls, while the high dose mean RF were significantly greater than controls with a 1.2-fold increase. At the 24 month sacrifice, the percent RF group means of the uterine tissue showed an exposure- related increase in all treated groups which was significantly increased but without a dose-relationship. Compared to the 12 month values, the mid and high dose values were increased by 24% at 24 months. (Table 29). The percent RF within the malignant mixed Müllerian (No. 379, killed at terminal sacrifice) of group 3 (5000 ppm) was 80. The RF within the two other associated Müllerian neoplasms in group 4 was 80 in No. 434 (with metastases in the kidney, ovary and lung, which died on week 105) and 90 in No. 436 (killed at terminal sacrifice). Mullerian tumours are rare in rats. It was noted by the notifier that Tamoxifen which is associated with risk of endothelial cancer in humans, causes an increase in RF across the endothelial layers in SD rats. The increase in RF is ≈ 3-fold. At 12 months, the magnitude of this increase by the high dose of iprovalicarb is ≈ 1.2 fold and ≈ 2 fold at 24 months.

Table 40: Comparison in female rats of benign and malignant neoplasms and the rate of proliferation in the uterus.

Group, - Dose (mg/kg/d) & Number (N)	Neoplasms: Rat Number	Neoplasms: Time of Observation (weeks)	RF within Neoplasm	RF Outside Neoplasm	Group Mean RF	Group Range RF
	12 Month Interim Sacrifice; Uterine Tissue [a]					
1. 0 (8)	NA	NA	NA	NA	38	26-48
2. 32 (10)	NA	NA	NA	NA	39	27-51
3. 326 (8)	NA	NA	NA	NA	39	35-45
4. 1380 (9)	NA	NA	NA	NA	45 *	37-54
	24 Month Terminal Sacrifice (TS)					
1. 0 (8)	NA	NA	NA	NA	29	19-35
2. 32 (8)	NA	NA	NA	NA	43 *	32-50
3. 326 (8)	379 **	TS, MA	80	40	45 * [b]	37-54
4. 1380 (8)	434 **	TS, MA	80	55	56 * [b]	51-66
	436 **	TS, MA	90	66	56 * [b]	51-66

RF, percent replicating cells taken from areas of highest PCNA positive incidence.
a composite of stromal (≈51%), luminal (48%) and myometrial (1%) cells.
b the mean value was obtained from animals with (outside the neoplasm) and without neoplasms.
NA, not applicable; NE, tissue could not be evaluated.
* Significant at $p<0.05$, in pair-wise comparison to control.
** not statistically significant (by pair-wise comparison or trend analysis).

Urothelial: In females, the urothelial cell (UC) proliferation values were similar to controls at 12 months of iprovalicarb exposure, in all three groups (Table 30). At the terminal euthanasia, the percent RF group mean of group 4 (20,000 ppm) of UB urothelial tissue showed a significant increase (40 with a range from 38-41) compared to controls (16 with a range 10-23) ($p<0.05$) (table 6.5.3.4) which cannot be explained directly. The percent RF within the 2 urothelial (adenomas) papillomas was 80 in No. 431, and 50 in No. 455, both killed at terminal sacrifice. It was concluded that there was increased diffuse proliferation after 12 months possibly resulting to the development of benign UC papillomas by 24 months.

Table 41. Comparison in female rats of benign (BE) and malignant (MA) neoplasms and the PCNA staining in bladder urothelium.

Group, Dose (mg/kg/d) & Number (N)	Neoplasms: Rat Number	Neoplasms: Time of Observation (weeks)	RF within Neoplasm	RF Outside Neoplasm	Group Mean RF	Group Range RF
	12 Month Interim Sacrifice; Urothelial Cells (Urinary Bladder) [a]					
1. 0 (7)	NA	NA	NA	NA	21	16 – 28
2. 32 (9)	NA	NA	NA	NA	22	19 – 26
3. 326 (10)	NA	NA	NA	NA	21	16 – 29
4. 1380 (9)	NA	NA	NA	NA	24	17 - 30
	24 Month Terminal Sacrifice (TS)					
1. 0 (7)	NA	NA	NA	NA	16	10 – 23
2. 32 (0)	NA	NA	NA	NA	TNE	TNE
3. 326 (0)	NA	NA	NA	NA	TNE	TNE
4. 1380 (5)	431	TS, BE	80	NE	40 * [a]	38 - 41
	455	TS, BE	51	41	40 * [a]	38 - 41

RF: percent replicating fraction taken from areas of highest PCNA positive incidence.
NA: not applicable; NE tissue could not be evaluated; BE, benign; TNE, tissue not examined.
[a] the mean value was obtained from animals with (outside the neoplasm) and without neoplasms.
* not significant at $p<0.05$ in a pair-wise comparison to group 1, requiring a level of significance of $p<0.01$.

Thyroid: The percent RF group mean of the nonneoplastic follicular thyroid tissue from all 3 exposure groups was similar to control (table 6.5.3.5). The percent RF within the follicular carcinoma of No. 424, killed at term, was 80. The RFs of follicular adenomas were PCNA negative for No. 425, killed at term and No. 473, killed moribund at 82 weeks, which also had an adenocarcinoma of the uterus, although the surrounding vascular elements in these animals were PCNA positive (Table 31).

Table 42. Comparison in female rats of benign (BE) and malignant (MA) neoplasms and the PCNA staining in thyroid.

Group, Dose (mg/kg/d) & Number (N)	Neoplasms: Rat Number	Neoplasms: Time of Observation (weeks)	RF within Neoplasm	RF Outside Neoplasm	Group Mean RF	Group Range RF
12 Month Interim Sacrifice – Thyroid Follicular Cells [a]						
1. 0 (10)	NA	NA	NA	NA	0.9	0.4 – 1.7
2. 32 (9)	NA	NA	NA	NA	0.9	0.5 – 1.3
3. 326 (8)	NA	NA	NA	NA	0.8	0.4 – 1.4
4. 1380 (9)	NA	NA	NA	NA	1.0	0.7 – 1.6
24 Month Terminal Sacrifice (TS)						
1. 0 (8)	NA	NA	NA	NA	1.8	1.1 – 2.7
2. 32 (8)	NA	NA	NA	NA	1.9	1.3 – 2.5
3. 326 (8)	390 *	TU, MA	90	1.6	1.8 [a]	1.4 – 2.8
4. 1380 (7)	424 *	TU, MA	80	2.5	2.0 [a]	1.4 – 2.5
	425 *	TS, BE	NE	1.7	2.0 [a]	1.4 – 2.5
	473 *	82. BE	NE	NE	2.0 [a]	1.4 – 2.5

RF, percent replicating fraction taken from areas of highest PCNA positive incidence;
NA, not applicable; NE, tissue could not be evaluated; TU, terminal euthanasia;
[a], the mean value was obtained from animals with (from outside the neoplasm) and without neoplasms;
*, trend analysis showed not significant at $p<0.05$, because the required trend analysis value should be $p<0.005$ for common tumor types (US FDA, 2000).

Table 43: Pertinent malignant and benign rat neoplasms (Males)

Group ID	***Rat No.***	***Weeks on Study***	***Neoplasm Diagnosis and Location (tissue)***
Group 1 Control Males	*10*	*107*	Hemangiosarcoma (MLN), C-cell adenoma (thyroid)
	23	*107*	Follicular adenoma (thyroid), adenoma (pituitary)
	48	*106*	Hemangiosarcoma (MLN), adrenomedullary adenoma (adrenal)
Group 2 500 ppm IPRO Males	*74*	*107*	Follicular adenoma (thyroid), adrenomedullary adenoma (adrenal)
	75	*101*	Fibrosarcoma (skin and other sites), malignant Schwannoma (stomach)
	79	*102*	Histiocytic sarcoma (systemic)
	107	*106*	Fibrosarcoma (stomach), C-cell adenoma (thyroid)
Group 3 5000 ppm IPRO Males	*113*	*61*	Fibrosarcoma (skin and other sites)
	123	*101*	Follicular adenoma (thyroid), malignant Schwannoma (heart)
	136	*91*	Follicular adenoma (thyroid), malignant neoplasm (adrenal medulla)
	161	*106*	C-cell carcinoma and adenoma (thyroid)
Group 4 20000 ppm IPRO - Males	*187*	*89*	Osteosarcoma (femur and knee joint) with lung metastases
	199	*101*	Chondrosarcoma (nasal cavity)
	203	*53*	Osteosarcoma (lower jaw)
	220	*94*	Osteosarcoma (femur and knee joint) with lung metastases
MLN, mesenteric lymph node.			

Table 44: Pertinent Malignant and Benign Rat Neoplasms (females)

Group ID	***Rat No.***	***Weeks on Study***	***Neoplasm Diagnosis and Location (tissue)***
Group 1 Control Females	*243*	*107*	Adenocarcinoma (uterus) with metastases in lung, MLN, brain and abdominal cavity
	250	*106*	Stromal sarcoma (uterus), adenocarcinoma (MG)
	260	*179*	Adenocarcinoma (uterus)
Group 2 500 ppm IPRO Females	*317*	*107*	Adenocarcinoma (uterus)
	334	*90*	Adenocarcinoma (uterus)
	340	*80*	Adenocarcinoma (uterus), C-cell adenoma (thyroid)
	343	*107*	C-cell adenocarcinoma and adenoma (thyroid)
Group 3 5000 ppm IPRO Females	*369*	*107*	Adenocarcinoma (uterus), adenocarcinoma (MG)
	379	*107*	Mixed Müllerian neoplasm (uterus), stromal polyp (uterus), luteoma (ovary)
	380	*106*	Follicular adenoma (thyroid), histiocytic sarcoma (systemic)
	390	*106*	Follicular carcinoma (thyroid)
	396	*106*	Adenocarcinoma (uterus)
Group 4 20000 ppm IPRO Females	*423*	*107*	Adenocarcinoma (uterus) with metastases in lung, liver, and pancreas, thecoma (ovary)
	424	*107*	Follicular carcinoma (thyroid), stromal polyp (uterus)
	425	*106*	Follicular adenoma (thyroid), stromal polyp (uterus)
	426	*53*	Adenocarcinoma (uterus)
	431	*107*	Papilloma (UB), thecoma (ovary), C-cell adenoma (thyroid)
	434	*105*	Mixed Müllerian neoplasm (uterus) with metastases in kidney, ovary and lung
	436	*106*	Mixed Müllerian neoplasm (uterus)
	453	*107*	Squamous cell carcinoma (uterus)
	455	*107*	Adenocarcinoma (uterus), papilloma (UB)
	457	*101*	Squamous cell carcinoma (clitoral gland)
	465	*105*	Adenocarcinoma (uterus)
	473	*82*	Adenocarcinoma (uterus), follicular adenoma (thyroid)
	475	*94*	Adenocarcinoma (uterus)
	476	*106*	Hemangiopericytoma and polyps (clitoral gland), C-cell adenoma (thyroid)
	478	*106*	Squamous cell carcinoma (clitoral gland), stromal polyp (uterus), C-cell adenoma (thyroid)
	480	*98*	Squamous cell carcinoma (uterus)
MLN, mesenteric lymph node; MG, mammary gland; UB; urinary bladder			

Table 45: Neoplasms and early deaths

Group ID	*Rat No.*	*Weeks on Study*	*Neoplasm Diagnosis and Location (tissue)*
4. 20000 ppm[b], female	*432*	*50*	Adenoma (pituitary)
2. 500 ppm, male	*148*	*50*	Malignant astrocytoma (brain)
4. 20000 ppm, male	*203*	*53*	Osteosarcoma (jawbone)
4. 20000 ppm, female	*426*	*53*	Adenocarcinoma (uterus)
4. 20000 ppm, male	*231*	*56*	C-cell adenoma (thyroid)
2. 500 ppm, female	*342*	*60*	Adenoma (pituitary)
2. 500 ppm, male	*65*	*60*	Benign granular cell neoplasm (brain)
2. 500 ppm, male	*113*	*61*	Fibrosarcoma (skin and other sites)
3. 5000 ppm, female	*414*	*64*	Malignant Schwannoma (skin and other sites)
2. 500 ppm, female	*314*	*65*	Liposarcoma (skin and other sites)
2. 500 ppm, female	*315*	*65*	Adenoma (pituitary)
4. 20000 ppm, female	*474*	*65*	Adenoma (pituitary)
3. 5000 ppm, female	*391*	*68*	Adenoma (pituitary)
3. 5000 ppm, female	*366*	*72*	Adenoma (pituitary)
2. 500 ppm, female	*333*	*72*	Adenoma (pituitary
3. 5000 ppm, male	*140*	*72*	Fibrohistiosarcoma (systemic)

a, During the first 18 months (2/3rds of study duration); b, IPRO.

Discussion:

Osteosarcoma: In male rats treated with doses of iprovalicarb (20000 ppm), 3 presented with osteosarcomas (Table 32). One animal (No. 203) had an osteosarcoma in the lower jaw, observed at 53 weeks, which contributed to the animal's demise due to its location, with an RF of 28. The second (No. 187, in the femur/knee joint with lung metastases) was observed at 89 weeks (PCNA negative), and the metastases contributed to its demise. The third (No. 220 in the femur with lung metastases) was observed at 94 weeks and had an RF of 38 These neoplasms occurred in high dose males, and not in high dose females, which received 20% higher dosing exposure of iprovalicarb for 2 years.

These osteosarcomas were considered by the dossier submitter to represent background neoplasms which are extremely rare and which occur at variable intervals, some around one year and some later. This rationale was also applied to the chondrosarcoma in the nasal cavity (No. 199, killed at 101 weeks) which was PCNA negative. This is also a very rare tumor. In addition, cartilage is not routinely sampled, which is why information on it is sparse in all data bases (Bomhard and Rinke, 1994; Poteracki and Walsh, 1998; Eiben and Bomhard, 1999; RITA, 1999; Giknis and Clifford, 2003).

Chemically induced osteosarcomas were recently described in rats at all dose levels, and were first detected around 20 months of exposure with subcutaneous injections of recombinant human parathyroid hormone (1-34) (Vahle et al, 2002; Hodsman et al, 2005). These osteosarcomas were associated with extensive clinical and morphometric changes throughout the 2 year study, which were not seen in the present study.

Uterus: The occurrence of malignant uterine neoplasms, especially the rare mixed Müllerian neoplasms, was associated with the mid dose (5,000 ppm, 1/48) and high dose (20,000 ppm, 2/50) of iprovalicarb, all 3 cases observed at the terminal sacrifice (Table 33). This rare background (2%) malignant neoplasm has only recently been adequately investigated. It occurs later in life in rats, i.e. at 23-25 months of age (Kaspareit-Rittinghausen and Deerberg, 1990) and metastases and displaces neighboring tissue and organs such as intestines and UB. In humans these tumours occur in younger patients (Williamson and Christopherson, 1972; Auerbach et al, 1988). The RF of the non-neoplastic uterine tissue was 39.7 for rat No. 379, 55.1 for rat No. 434, and 66.2 for rat No. 436. Within the neoplasms the RFs were 80, 80, and 90, respectively. The mean group RF of the non-neoplastic tissue of all 3 dose levels was significantly increased (Table 29). Neoplasms occurred only in groups 3 and 4.

Urothelial: There was an increase in the incidence, although not statistically significant, of benign transitional (urothelial) cell papillomas of the UB in two high dose female rats (Nos. 431 and 455, both at 107 weeks) (Table 30). The individual RF of each of these neoplasms was high both within and outside the neoplasm. In addition, the group mean RF was significantly increased compared to controls. This increase in RF could not be explained directly, but it may be associated with iprovalicarb which is excreted in the urine. The dossier submitter considered that these 2 benign neoplasms were of questionable relevance to humans because there are major intraluminal differences between rats and humans and it is unlikely that the production of bladder neoplasia in rats by a non-genotoxic mode of action would be predictive of cancer hazard to humans (Iatropoulos et al, 1994; Williams et al, 1996; Cohen, 1998; Rice et al, 1999, Cohen et al, 2004).

Thyroid: Thyroid follicular cell combined (benign and malignant) neoplasia was significantly increased as a positive trend in high dose females. These neoplasms are uncommon in Wistar rats, but are known to occur late in life (RITA, 1999; Giknis and Clifford, 2003). The group RF mean of the nonneoplatic follicular thyroid tissue was comparable across all study groups (Table 31). The individual RF value of non-neoplastic areas was comparable to control values, whereas the RF within the follicular carcinomas was 80-90 (No. 390, group 3 at 106 weeks, or No. 424, group 4 at 107 weeks).

Conclusion

PCNA analysis of the 12 and 24 month sections of all tumour bearing tissues (bone/males and thyroid, urinary bladder, uterus/females) from the two year rat study were reported. A slight increase (pairwise $p<0.05$) in RF was seen in all treated bone samples at 12 months. No RF values were obtained for the 24 month samples as sections other than two osteosarcomas were PCNA negative. No conclusion can be made from this data other than an indication for a marginal proliferative response on 12 month bone tissue. In the uterus, a significant increase (1.2-fold; $p<0.05$) in RF value was seen for the high dose at 12 months. At 24 months, the RF was significantly increased in all treated groups without a clear dose-relationship. The RF value at the high dose was 2-fold greater than the control. The RF values in rat urothelial cells from the female 12 month sacrifice were all similar to controls, while the RF value of the high dose group at the 24 month sacrifice showed a significant increase. The percentage RF value

in non-neoplastic female thyroid tissues from both 12 and 24 month sections were not different from controls. High RF values were recorded for all tumour tissues analysed.

According to the authors, the mean value of the dosed group (-SD) should be at least ≥ 3 times greater than the mean control value (+SD) to achieve biological significance. This was not achieved in any tissue samples other than the tumour sections for each tissue (other than bone). Statistically significant increases in RF were recorded in a number of tissues, however, the biological significance of the observed increases in RF are difficult to assess. Overall, the assay does not contribute greatly to the understanding of the possible tumourigenicity or otherwise of iprovalicarb.

Other relevant information

The results of the special metabolism study and the associated biokinetics study, as well as those of the bioavailability study with iprovalicarb show a number of differences that occur following administration of the 20000 ppm dose relative to the situation at lower doses. As the administered dose increases, so too does the amount of unchanged parent material (both in the plasma and in faeces). At high doses also, the isomeric ratio of the main metabolite (M03; iprovalicarb carboxylic acid) shifted in favour of the SR diastereomer. However, the principal metabolic pattern did not change i.e. the metabolites identified and the isomeric ratio of the parent compound. It appears that with exposure to high doses of test substance, the capacity of the liver to metabolise the parent compound is impaired. There is an apparent 'high dose' effect in operation.

The standard battery of mutagenicity/genotoxicity assays carried out with iprovalicarb gave consistently negative results. There was, therefore, no evidence of any genotoxic potential. An additional ^{32}P post-labelling assay *in vivo* was performed, however, in the uterus and urinary bladder of female Wistar rats to investigate whether there was any indication of specific genotoxic action in these organs. The formation (or otherwise) of treatment-related DNA adducts in these tissues was observed only in positive control groups. Results with iprovalicarb were negative, which further strengthens the case for the lack of genotoxic potential of iprovalicarb. However, only nine animals were tested and it could be argued that this test is therefore not sensitive enough to pick up precursor events that may be related to rare tumours of the type observed in the chronic rat study.

There is information in the literature to support the theory that a close statistical correlation exists between the occurrences of 'foci of altered hepatocytes' as precursors of liver cell tumours. A liver foci test (Enzmann, 1993, DAR/RAR B.6.8.2.5) designed as a short-term carcinogenesis assay to detect an initiation potential of a substance was performed using iprovalicarb. Iprovalicarb was administered by gavage to groups of 10 male rats (Bor:WISW) at concentrations of 0 (vehicle only) and 1000 mg/kg (in a suspension of Cremophor EL/demineralized water 2% v/v) for 28 days, followed by eight weeks of promotion treatment with phenobarbital (0.05%). No initiation potential of iprovalicarb could be detected in the liver in this test under the study conditions employed.

4.10.4 Summary and discussion of carcinogenicity

Rat: In the chronic rat study, treatment-related effects were noted in females at 5,000 ppm and in males at 20,000 ppm. Body weight was decreased (8%) in males and (12%) in females of the 20,000 ppm group, and (7%) in females of the 5,000 ppm group. Changes in liver

morphology and function, beginning at 5,000 ppm, were predominantly notes in females. Increased cholesterol, decreased ASAT, and decreased total bilirubin levels, increased relative liver weights and increased incidences of hepatocellular hypertrophy were observed in females at 5,000 ppm. These can be considered to be adaptive responses resulting from the inducing effect on microsomal enzymes, with corresponding slight effects on lipid metabolism.

The histopathological findings indicate a shift in the incidences of certain tumours at 5,000 ppm (mixed Muellerian uterine tumours and thyroid follicular cell adenoma/carcinoma in females), and at 20,000 ppm (transitional cell papillomas of the urinary bladder in females, and skeletal system tumours in males). None of these tumour types was seen in control animals, and are only rarely observed in long-term studies. Although the incidences of these tumours were low, they are all either extremely rare or uncommon in Wistar rats. Data from a 32P-postlabelling assay performed with uterine tissue and urinary bladder epithelium in vivo gave no indication of DNA-adduct formation. A PCNA assay on neoplastic and non-neoplastic tissues from tumour-bearing tissues in the rat study indicated some increases in RF values in non-tumour tissues, possibly consistent with increased proliferation in these tissues. These increases were considered by the authors to be non-biologically relevant. The relevance of these findings to the assessment of the carcinogenicity of iprovalicarb was difficult to assess.

Therefore, although some mechanistic data was provided which suggest that iprovalicarb is not a tumour initiator, no mode of action could be established for induction of any tumour type and possible relationship to treatment could not be excluded. It should be noted that the animals concerned were all subjected to extremely high doses (1379.7 mg/kg bw for females and 1109.6 mg/kg bw for males), as the test substance was observed to be of very low general toxicity in subchronic studies and there was no evidence of genotoxicity in the adequate data submitted.

Mouse: In the long-term mouse study, food and water intakes in the high dose group males were slightly elevated, while body weight was slightly depressed. There was evidence of a slightly elevated blood urea level in both sexes at 1,400 ppm and above, indicating a minor impairment in kidney function. There was also a reduction in absolute and relative kidney weights which might correlate with the marked decrease in the incidence of tubular vacuolisation in kidneys of the male animals at 1,400 and 7,000 ppm. No histopathological kidney changes were observed in any of the females

Elevated triglyceride levels were found in high-dose males only. Hepatocellular lesions, described as “clear-cut, apparently empty intracellular vacuoles, in single or groups of liver cells distributed in the parenchyma” were observed at an increased incidence in males (only) of the 7,000 ppm dose group. These appear to represent liver cell degeneration and are regarded as an adverse effect.

Data relevant to carcinogenicity classification

An increased incidence of rare and uncommon tumours was recorded following chronic exposure of Wistar rats to iprovalicarb. At 20000 ppm (1109.6/1379.7 mg/kg bw/day), males developed malignant osteosarcoma and benign transitional cell papillomas of the urinary bladder were increased in females. At the mid dose (5000 equiv to 262/326 mg/kg bw/day) and the high doses, females developed malignant mixed Muellerian tumours of the uterus and follicular cell tumours of the thyroid gland. There was no evidence of carcinogenicity in the long-term mouse study (0, 280, 1400, 7000 ppm (58/97, 283/503, 1566.8/2544 mg/kg bw/day).). Tumours occurred at a number of apparently unrelated sites, in one species only and at high doses, not otherwise considered excessively toxic. Iprovalicarb is not genotoxic. There is no

evidence of pre-neoplastic change in any of the tumour-bearing organs and although some mechanistic data was presented, no mode of action can be supported. There was no evidence of carcinogenicity in the second species tested.

The data are considered 'limited' due to these considerations, however, a possible relationship to treatment cannot be discounted and all tumours have at least some relevance to humans.

4.10.5 Comparison with criteria

CLP: Regulation (EC) No. 1272/2008

The criteria for classification as a carcinogen under the CLP Regulation are as follows;

1. **Category 1:** Known or presumed human carcinogens. A substance is classified in Category 1 for carcinogenicity on the basis of epidemiological and/or animal data. A substance may be further distinguished as:
2. **Category 1A**, known to have carcinogenic potential for humans, classification is largely based on human evidence, or
3. **Category 1B**, presumed to have carcinogenic potential for humans, classification is largely based on animal evidence.

Differentiation between classification in Category 1A and 1B is based on strength of evidence together with additional considerations. Iprovalicarb does not fit the criteria for Cat 1A or B as there is no human evidence and the animal data are limited for a number of reasons.

Category 2 Carcinogen : Suspected human carcinogen.

"Substances are classified as Category 2 Carcinogens when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. Such evidence may be derived either from limited evidence of carcinogenicity in human studies or from limited evidence of carcinogenicity in animal studies."

In addition to the determination of the strength of evidence for carcinogenicity, a number of other factors need to be considered that influence the overall likelihood that a substance poses a carcinogenic hazard in humans. Some important factors which may be taken into consideration, when assessing the overall level of concern are:

- tumour type and background incidence;
- multi-site responses;
- progression of lesions to malignancy;
- reduced tumour latency;
- whether responses are in single or both sexes;
- whether responses are in a single species or several species;
- structural similarity to a substance(s) for which there is good evidence of carcinogenicity;
- routes of exposure;
- comparison of absorption, distribution, metabolism and excretion between test animals and humans;
- the possibility of a confounding effect of excessive toxicity at test doses;

According to the criteria, a substance is classified as a Category 2 Carcinogen when evidence is obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in Category 1A or 1B, based on strength of evidence together with additional considerations. In the case of iprovalicarb, there is limited evidence of carcinogenicity in animal studies in which there was an apparent increase in rare and uncommon tumours following chronic exposure of Wistar rats to iprovalicarb. Category 2 is favoured over Category 1 since the effect is confined to a single species at high doses. As described above, the increased incidences at the high dose of malignant osteosarcoma (males) and benign transitional cell papillomas of the urinary bladder (females) and malignant mixed Muellerian tumours of the uterus from the intermediate dose, were outside the concurrent controls and the historical control data ranges provided. The observed statistically significant trend towards an increase in thyroid follicular adenoma in females was outside the relevant historical control range given, and relationship to treatment cannot be disregarded. There is no evidence of genotoxicity. No mechanisms are proposed for the observed tumours and all tumours have at least some relevance to humans. The evidence is therefore 'limited' and classification with Carc. 2 is proposed as the most appropriate in this case.

4.10.6 Conclusions on classification and labelling

Rare tumours were increased in four different organs in an apparent treatment-and dose-related manner in the rat. Carc. 2 is recommended on the basis of limited evidence for a carcinogenic potential from a single species exposed long-term to significantly high doses of iprovalicarb.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS proposed to classify iprovalicarb as carcinogenic category 2 on the basis of the increases in the incidences of the following tumours: mixed Muellerian tumours in the uterus, follicular cell adenomas and carcinomas in thyroid, transitional cell papilloma in urinary bladder, squamous cell carcinomas in clitoral gland and osteosarcoma in femur and other bones.

Comments received during public consultation

Six different MSCAs supported the DS's proposal for classification of iprovalicarb as carcinogenic category 2.

Assessment and comparison with the classification criteria

Study 1: Chronic toxicity and carcinogenicity investigations in Wistar rats (RAR/RAR B.6.5.1)

Groups of 50 male and 50 female Wistar rats each were administered iprovalicarb (95.8-98.5% purity) *ad libitum* in their diet at concentrations of 0, 500, 5000, or 20000 ppm for 24 months. Ten additional rats/sex and dose were included in the study for an interim

sacrifice after 12 months of treatment. The study conformed to the requirements of OECD TG 453 with no deviations.

There was no evidence of a substance related effect on mortality. Table 5 shows the most remarkable non-neoplastic findings that were comparable to effects reported in other oral repeated dose toxicity studies of shorter duration and can be summarised as follows: marginal reductions of body weights, clinical chemistry alterations, higher relative liver weights and increased incidence of hepatocellular hypertrophy at doses of 5000 ppm and higher. Table 9 displays the main neoplastic findings found in this study. Table 10 displays several historical control data (HCD) provided in the CLH report for the tumours reported in the table 9.

***Table 9**: Neoplastic changes induced by iprovalicarb in animals at terminal sacrifice in the carcinogenicity studies in Wistar rats (RAR/RAR B.6.5.1).*

	Sex:	**Male**				**Female**			
	Dose (ppm):	**0**	**500**	**5000**	**20000**	**0**	**500**	**5000**	**20000**
Urinary bladder	No. exam:	50	50	49	50	50	49	48	50
Papilloma, Transitional Cell	b	0	0	0	0	0 t*	0	0	2 (4) ns
Mammary gland	No. exam:	50	50	49	49	50	49	48	50
Fibroadenoma	b	0	0	0	1	8	6	11	3
Adenoma	b	0	0	0	0	0	1	0	0
Adenocarcinoma	m	0	0	0	0	6 t**	3	2	0$
Uterus	No. exam:	-	-	-	-	50	50	48	50
Adenocarcinoma	m	-	-	-	-	2	3	3	6 ns
Mixed Muellerian tumour	m	-	-	-	-	0 t*	0	1	2 (4) ns
Pituitary gland	No. exam:	50	50	49	50	50	50	48	50
Adenoma / Pars Distalis	b	11	6	10	12	20	19	20	14
Thyroid gland	No. exam:	50	50	49	50	50	50	48	50
Adenoma / Follicular Cell	b	1	1	2	0	0 t*	0	1	2 (4) ns
Carcinoma / Follicular Cell	m	0	0	0	0	0	0	1	1 (2)
Skeletal system	No exam:	50	50	49	50	50	50	48	50
Osteosarcoma									
-Femur/knee joint	m	0 t*	0	0	2 ns	0	0	0	0
-Bone, other site#					1				
-total		0	0	0	3 (6)	0	0	0	0
Chondrosarcoma (Nasal cavity)#	m	0	0	0	1	0	0	0	0
Clitoral gland#	No exam:	-	-	-	-	50	50	48	50
Carcinoma/Squamous Cell	m	-	-	-	-	0	0	0	2 (4)

NOTE: *the incidence of each type of lesion and their percentage (in brackets) are shown.*
 # = organ / tissue not routinely examined;
t and t** = p < 0.05 or p < 0.01 respectively, in trend test.*
** = p < 0.05 in one-tailed pairwise group comparison.*
$ = no pairwise group comparison performed;
ns = not significant; b = benign; m = malignant.

Urinary bladder

Transitional cell papilloma (benign) was found in the urinary bladder of 2 females (4%) dosed with 20000 ppm. This incidence was statistically significant in a trend test analysis ($p < 0.031$). There were no neoplastic findings in the bladder of males. Increased incidences of hyperplastic/pre-neoplastic lesions such as focal or diffuse hyperplasia were not observed in either sex. There were no transitional cell carcinomas observed in the female rats and no transitional cell adenomas or carcinomas in the male rats in this study. Transitional cell papilloma in females is recorded in the RITA database with a spontaneous frequency between 0-2.2%. Thus, the study incidence for this rare urinary bladder tumour exceeded the relevant historical background incidence for both this laboratory (0-2.0%) and the extended RITA database.

Mammary gland

There were several incidences of different types of tumours in mammary gland. This incidence of adenocarcinoma was statistically significant in a trend test analysis ($p < 0.01$). However, no dose-response could be established for any of the 3 different tumours (fibroadenoma, adenoma and adenocarcinoma). The lack of dose-response, the high incidence of controls and the lack of historical control data for comparison induces RAC to consider these tumours as non-relevant for classification purposes.

Uterus

Malignant mixed Muellerian tumours in the uterus were observed in one of the 5000 ppm female and in two 20000 ppm females. In one high dose animal, metastases of this tumour type had occurred in several organs. Trend test analysis was statistically significant ($p = 0.038$) for this lesion while a dose adjusted pairwise group comparison did not achieve statistical significance. These tumours are rare in rats and are postulated to originate from pluripotent mesodermal cells of the Muellerian duct. They are characterised by an admixture of malignant epithelial and mesenchymal components. In this study, the epithelial structures consist of glandular, squamous, or anaplastic epithelium, while the mesenchymal parts differentiate towards osteosarcoma and/or chondrosarcoma. The incidence of this tumour exceeded the highest historical control incidence rate in the updated in-house laboratory historical control database (0%) and the RITA database (2%). Mixed Muellerian tumours occurred in 6 cases/6004 animals (0.1%) in the complete RITA database (1984-2009) and in 3/3585 (0.08%) in studies conducted within 5 years of this study. Spontaneous frequencies per study vary between 0 and 2% (RITA database). Adenocarcinoma incidence was slightly higher in the 20000 ppm group than in the other treatment groups, but the difference was not significant.

Pituitary gland

There were several incidences of adenoma pituitary gland. However, no dose-response could be established and therefore RAC does not consider these tumours associated to the treatment and therefore cannot be considered for classification purposes.

Thyroid gland

Pre-neoplastic lesions (focal hyperplasia of the follicular epithelium) were seen in similar incidences in all groups. Other lesions indicating an effect on the thyroid gland such as hypertrophy were not reported for iprovalicarb.

A non-statistically significant increase in the incidence of follicular cell adenoma was observed in thyroid glands of females at 5000 and 20000 ppm. There was a significant positive trend in the incidence of adenomas ($p = 0.03$). The incidence of this tumour exceeded the average

of the in-house laboratory historical control database (average 0.6%) with a range of 0-2% and some other HCD (table 10). However, the highest HCD for the extended RITA database was 6.1% that was lower than the incidence reported in this study.

An increased incidence in follicular cell carcinoma (2%) was observed in thyroids of 20000 ppm females which exceeded the highest historical control rate of the in-house database (0%) but did not exceed the highest historical control incidence rate (5%) registered in the RITA data base. The incidence of follicular cell adenoma also exceed the average of some other HCD, although were slightly minor than the highest value of the range (table 10).

The incidence of thyroid follicular cell adenoma, adenocarcinoma and adenoma/carcinoma combined in female rats in the iprovalicarb rat study is therefore outside the relevant historical control incidence.

Skeletal system

Three males of the 20000 ppm group, which died or had to be killed in a moribund state, were diagnosed with malignant tumours of the skeletal system; 2 metastasising osteosarcomas of the femur, 1 osteosarcoma of the lower jaw, and 1 chondrosarcoma of the nasal cavity. Spontaneous osteosarcoma is uncommon in both laboratory animals and man and particularly rare in rats. Chondrosarcoma and osteosarcoma are considered to have a common aetiology but are not considered together as the nasal cavity is not normally examined. The incidence of osteosarcoma of the bone observed in male rats (6%) exceeded the highest historical control incidence rate in both the updated in-house laboratory database (average 0.1% (range 0.0-1.7%) in males and 0.0% in females) and the RITA historical control database for males (0.2% (0.0-2%)) and females (0.1% (0.0-4.0%)). There were no control data available for chondrosarcomas.

Clitorial gland

Two females of the 20000 ppm group had a squamous cell carcinoma of the clitoral gland ($p = 0.089$). Clitoral glands are not protocol organs and are not normally examined in carcinogenicity studies, therefore, lesions not seen at necropsy would not be detected. Spontaneous clitoral gland tumours are infrequent in rats. The incidence rate in two other studies where this gland was examined in the test laboratory was 2.4-3.0% (2 studies) and in the extended RITA database was 0.5% (range of 0.0-4.3%). The biological significance of this finding cannot be assessed in the absence of histological examination of this gland in all other high dose animals in the study.

Table 10: *HCD from different sources for tumours reported in the table 9*

	Urinary bladder		Uterus	Thyroid gland		Skeletal system		Clitoral gland
	male	female	MMT	Adenoma	Carcinoma	male	female	female
Bayer								
All	0	0.1 (0.0-2.0)	0.1 (0.0-1.7)	0.6 (0.0-0.2)	0.0	0.1 (0.0-1.7)	0	
1993-2003		0	0.1 (0.0-1.7)					2.3-3.0
RITA	0.7 (0.0-15)	2.2 (0.0-2.2)	0.1 (0.0-2.0)	0.0-6.1	0.0-5.0	0.2 (0.0-2.0)	0.1 (0.0-4.0)	0.5 (0.0-4.3)

Bomhard and Rinke 1994[3]	Male: 1.1 (0.0-4.4) Female: 0.9 (0.0-6.5)	Male: 0.5 (0.0-2.2) Female: 0.2 (0.0-2.1)
Eiben and Bomhard, 1999[4]	Concluded a somewhat decreasing trend but without statistical significance	
HED 0050652[5]	Male: 0.7 (0.0-4.0) Female: 0.6 (0.0-2)	Male: 0.1 (0.0-2.0) Female: 0
Carlus *et al.* 2013[6]	Male: 0.4 (0.0-2.0) Female: 0.2 (0.0-1.7)	Male: 0.6 (0.0-1.7) Female: 0.4 (0.0-2.0)
Poteracki and Walsh, 1998[7]	Male: 3.9 (1.7-6.9) Female: 2.8 (2.0-3.3)	Male: 0.9 (0.0-1.7) Female: 1.5 (0.0-3.3)

Bayer/Pharma all contains data from up to 22 studies dated between 1986 and 2007. Bayer/Pharma 1993-2003 contains data from up to 13 studies dated on these years. RITA contains data from up to 119 studies dated from 1984 to 2009. Figures are shown in percentage. RAC based the assessment only in in house HCD (Bayer 1993-2003). MMT = Mixed Muellerian uterus tumour.

Supplementary study: Proliferating cell nuclear antigen (PCNA) immunohistochemical evaluation report on selected target tissues from SZX072 (Iprovalicarb)

In order to monitor the rate of proliferation in possible tissues targeted in study 1 (RAR/RAR B.6.5.1) proliferating cell nuclear antigen (PCNA) immunohistochemical stain was performed on bone and cartilage tissues in males and on urinary bladder, uterus and thyroid tissues in females from archival material (blocks) from the 24 months terminal sacrifices from 8 randomly selected samples per group of rats, including the samples with neoplasms. Subsequently, an additional 10 rats from the 12 months interim segment of the study were evaluated.

A slight increase (pairwise $p < 0.05$) in replicating fraction (RF) was seen in all treated bone samples at 12 months. No replicating fraction values were obtained for the 24 month samples as sections other than two osteosarcomas were PCNA negative. No conclusions can be made

[3] Bomhard and Rinke, Frequency of spontaneous tumours in Wistar rats in 2-year studies. Exp. Toxicol. Pathol. 1994; 46: 17-29.

[4] Eiben, and Bomhard, Trends in mortality, body weights and tumour incidences of Wistar rats (Bor:WISW (SPF Cpb – bred Winkelmann, Borchen, Germany) over 20 years. Exp. Toxicol. Pathol. 1999; 51: 523-536.

[5] HED 0050652. Cancer Assessment Document: Evaluation of carcinogenic potential of iprovalicarb, April 2002. US EPA CARC HED OPP.

[6] Carlus *et al.,* Historical control data of neoplastic lesions in the Wistar Hannover Rat (RjHan:WI – bred in Janvier, France) among eight 2-year carcinogenicity studies. Exp. Toxicol. Pathol. 2013; 65: 243-253.

[7] Poteracki and Walsh, Spontaneous neoplasms in control Wistar Rats: A comparison reviews. Toxicol. Sci. 1998; 45: 1-8.

from this data other than an indication for a marginal proliferative response on 12 months bone tissue.

In the uterus, a significant increase (1.2 fold; $p < 0.05$) in replicating fraction value was seen for the high dose at 12 months. At 24 months, the replicating fraction was significantly increased in all treated groups without a clear dose-relationship. The replicating fraction value at the high dose was 2-fold greater than the control.

The replicating fraction values in rat urothelial cells from the female 12 months sacrifice were all similar to controls, while the RF value of the high dose group at the 24 month sacrifice showed a significant increase. The percentage replicating fraction value in non-neoplastic female thyroid tissues from both 12 and 24 month sections were not different from controls. High replicating fraction values were recorded for all tumour tissues analysed.

According to the authors, the mean replicating fraction of the dosed group should be at least ≥ 3 times greater than the mean replicating fraction control value in order to achieve biological significance. This was not achieved in any tissue samples other than the tumour sections for each tissue (other than bone). Statistically significant increases in replicating fraction were recorded in a number of tissues, however, the biological significance of the observed increases in replicating fraction are difficult to assess. Overall, the assay does not contribute greatly to the understanding of the possible tumourigenicity or otherwise of iprovalicarb.

Study 2: Oncogenicity study in B6C3F1 Mice

Iprovalicarb (95.8-98.5%) was administered via the food to groups of 50 male and 50 female B6C3F1 mice at concentrations of 0, 280, 1400, or 7000 ppm over a period of up to 105 weeks. Ten additional mice/sex/dose were treated likewise and were sacrificed after 52 weeks. The study was conducted in accordance with OECD TG 451 without significant deviations.

There was no evidence of a substance-related effect on mortality. Table 5 shows the most remarkable non-neoplastic findings that were comparable to effects reported in other oral repeated dose toxicity studies of shorter duration and can be summarised as follows: marginal reductions in body weights, clinical chemistry alterations, alterations in absolute and relative kidney weights and increased incidence of fatty change in liver. Table 11 displays the main neoplastic findings found in this study.

There was no evidence that the tumours (benign and/or malignant) which were recorded throughout the study (including in the mice which died intercurrently) were treatment-related. A higher incidence of hepatocellular neoplasms was recorded in males at 280 ppm in comparison with controls or other treated males (incidence of hepatocellular adenoma plus carcinoma in ascending order of dose: 28%, 50%, 26%, 28%), indicating no dose response relationship. No remarkable increase in the incidence of hepatocellular neoplasms was observed in the females up to and including 7000 ppm. As the B6C3F1 mouse strain is known for its relatively high incidences of hepatocellular neoplasms ranging from 7% to 58% in control males, and the incidence of hepatocellular neoplasms does not show a dose response relationship, the increased number of liver tumours in males at 280 ppm cannot be linked to treatment with iprovalicarb. There was no evidence of any other increase of tumour incidences associated with treatment.

Table 11: *Neoplastic changes induced by iprovalicarb in animals at terminal sacrifice in the carcinogenicity studies in B6C3F1 mice*

		0 ppm	280 ppm	1400 ppm	7000 ppm
No. of examined animals		50	50	50	50
Liver adenomas	males	7 (14%)	15 (30%)	7 (14%)	7 (14%)
	females	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Liver carcinomas	males	7 (14%)	10 (20%)	6 (12%)	7 (14%)
	females	2 (4%)	1 (2%)	1 (2%)	0 (0%)

Comparison with criteria

The histopathological findings indicate a shift in the incidences of certain tumours at 5000 ppm (mixed Muellerian uterine tumours, thyroid follicular cell adenomas and carcinomas in females), and at 20000 ppm (transitional cell papilloma of the urinary bladder and squamous cell carcinoma in clitoral gland in females and osteosarcoma in femur and other bones of males in addition to those reported at 5000 ppm). None of these tumour types were seen in control animals, and are only rarely observed in long-term studies. Although the incidences of these tumours were low, they are all either extremely rare or uncommon in Wistar rats. Thus, iprovalicarb seems to be a substance with multiple targets for carcinogenicity and might be potentially considered for classification within category 1B, especially taking into consideration that these tumours appeared in animals without significant systemic toxicity.

However, RAC notes several issues that notably reduce the concern as:

- Iprovalicarb is not genotoxic *in vivo* or *in vitro.*
- The reported tumours in rats appeared at very high doses of 1110 mg/kg bw/day in males and 1380 mg/kg bw/day in females (the difference might explain the higher incidence of tumours in females than in males).
- The incidences of all these types of tumours were always low (a maximum of 4%) despite the doses well above the typical dose of 1000 mg/kg bw/day considered as limit dose.
- No evidence of pre-neoplastic changes was found.
- The carcinogenesis seems to be confined to a single species (rats) because doses as high as 2544 mg/kg bw/day in mice did not cause any treatment-related increases in neoplastic lesions.

In conclusion, due to the above stated reasons RAC considers that the evidences of human carcinogenicity are limited and therefore supports the DS's proposal for **classifying iprovalicarb as carcinogenic category 2 (H351; suspected of causing cancer).**

4.11 Toxicity for reproduction

Table 46: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Developmental toxicity, Wistar rat AS: 95.8% pure 0, 100, 300 or 1000 mg/kg bw	No effects seen up to and including the limit dose of 1000 mg/kg bw.		Stahl, B., 1996 DAR/RAR B.6.6.2.1

Developmental toxicity, Russian rabbit AS: 95.8% pure 0, 100, 300 and 1000 mg/kg bw	No effects seen up to and including the limit dose of 1000 mg/kg bw.		Kolb, J., 1995 DAR/RAR B.6.6.2.2
Two generation reproduction, Wistar rat AS: 99.2 % 0, 100, 2000 or 20000 ppm (mg/kg bw equivalent ranges over 2 generations: 7.3-10.8, 146.3-239.5, 1514.3-2944.1 mg/kg bw).	Parental/reproductive NOAEL: 2000 ppm (≈146.3 mg/kg bw): Reduced body weight in F1 and F2 pups during lactation, reduced mean litter weight at weaning in F1 pups, an increase in relative liver weights in F2 weanlings, and a reduced lactation index in F1 pups at 20000 ppm.		Eiben, R., 1997 DAR/RAR B.6.6.1

4.11.1 Effects on fertility

4.11.1.1 Non-human information

Study 1: Two generation study in Wistar rats. DAR/RAR B.6.6.1

Groups of 30 male and 30 female Wistar (strain ICO:WU (IOPS Cpb) rats were administered iprovalicarb (99.2%) at dietary concentrations of 0, 100, 2000 or 20000 ppm over 2 generations in a OECD Guideline compliant study.

Findings:

Food and substance intake: In F_0 and F_1 parents during premating, gestation and lactation, the feed intake per animal and per day did not differ from the control data to a toxicologically relevant extent in treated males and females up to 2000 ppm. In the 20000 ppm group, F_0 and F_1 females ingested 14.7% and 23% per kg bw, respectively, more feed than controls during the premating period. Due to the slight increase in feed consumption and partly (males) lower body weights at 20000 ppm, males and females of this group had a somewhat higher test substance intake than that expected from the dosing factor (Table 47).

Table 47: Test substance intake of F_0 and F_1 parents

F_0 Mean daily intake of test substance during the premating period				**F_1** Mean daily intake of test substance during the premating period			
Dose (ppm)	**Sex**	**Weeks**	**mg/kg bw per Day**	**Dose (ppm)**	**Sex**	**Weeks**	**mg/kg bw per Day**
100	m	10	7.3	100	m	15	7.7
2000	m	10	146.3	2000	m	15	155.3
20000	m	10	1514.3	20000	m	15	1838.0
100	f	10	9.6	100	f	15	10.8
2000	f	10	190.4	2000	f	15	239.5
20000	f	10	2074.0	20000	f	15	2944.1

Mortalities/Clinical signs: There is no evidence of treatment-related mortality in male or female F_0 and F_1 animals at levels of up to 20000 ppm and no test substance-related effects on the appearance or behaviour were observed.

Body weight: The body weights of the male and female F_0 and F_1 animals receiving 100 or 2000 ppm did not differ significantly from those of controls. In the 20000 ppm group, males of the F_0 generation exhibited slightly reduced body weights (maximally 6%, $p<0.05$) from week 13 onwards, while the F_1 generation males had significantly depressed body weights (approx. 10% in week 13). In 20000 ppm females of the F_0 generation, a slight to moderate body weight depression was detected during premating (< 5%), and lactation (up to 7%) period. In the F_1 females, the body weight reduction reached statistical significance only during week 13 and on lactation day 14.

Table 48: Body weights during lactation (female F_0 and F_1)

		Female body weight (g) on lactation day					
Dose level (ppm)		**0**	**4**	**7**	**14**	**21**	**28**
0	**F_0**	237	252	257	263	262	239
	F_1	265	272	282	290	283	265
100	**F_0**	236	251	260	265	260	242
	F_1	273	281	288	295	293	277
2000	**F_0**	230	239	248	255	249	236
	F_1	268	278	284	288	290	269
20000	**F_0**	224	236^{+}	241^{+}	249	243^{+}	238
	F_1	251	263	269	275^{+}	271	265

U-test: + = 5% significance level.

Parental:

Pathology: No significant gross pathological or histopathological findings were made at necropsy of male or female F_0 and F_1 animals at doses of up to 20000 ppm. No noticeable discrepancies between implantation sites and number delivered pups occurred in any of the treatment groups. Post implantation loss was comparable in treated and untreated rats.

Organ weights: In F_0 parents, the weights of the liver and testes were comparable with those of the controls up to 2000 ppm. In the 20000 ppm group, higher relative liver weights were recorded in males (14%) and in females (22.2%). In the 20000 ppm males, relative testes weights were elevated by 10%.

In F_1 parents, the absolute (males only) and relative (males and females) liver weights were significantly elevated. Relative weights were raised by 11.4% and 28.3% in males and females respectively. Other organ weights (including kidney weights determined in F1 rats) showed no deviation between treated and untreated groups that were dose-dependent.

Table 49: Mean absolute and relative liver weights.

	Male				**Female**			
Dose level (ppm)	**0**	**100**	**2000**	**20000**	**0**	**100**	**2000**	**20000**
Absolute liver weights								
F_0	15133	15740	15023	16340^{+}	10048	9680	10204	12230^{++}
F_1	17874	18601	17351	18521	10481	10677	11294	13363^{++}
Rel. liver wt. [mg/100 g bw]								
F_0	3265	3338	3285	3713^{++}	4206	4025	4252	5142^{++}
F_1	3422	3507	3353	3811^{++}	3936	3870	4187	5051^{++}

Histopathology: In both generations, there were no treatment-related alterations of liver morphology in the 100 ppm group. However, in both the 2000 and 20000 ppm groups, a majority of rats exhibited minimal to slight cytoplasmic changes in the hepatocytes.

Spermatological evaluations: In F_0 males, a slight reduction in initial (at 1 minute) sperm motility was detected in the 20000 ppm group. However, all but one (out of a total of 15 males) was found to be fertile. There was no dose-related effect on sperm motility. Some sperm abnormalities (head-tail break, head-tail separation or sharps in the tail) were found more frequently at 20000 ppm. However, this is mainly due to a high head-tail separation incidence in one particular animal. In F_1 males, no effect was detected on any sperm parameters at 20000 ppm. Therefore, males of the 100 and 2000 ppm were not examined in this respect.

Oestrus cycle length: No significant change in either generation.

Reproductive parameters: Fertility indices were unaffected by treatment. There were no effects on litter size, pup weights, sex ratio or on viability of these pups up to day 4 p.p. Mating performance of the F_0 or the F_1 animals was not affected by the treatment at levels of up to 20000 ppm.

Table 50: Summary of fertility and offspring findings.

	Gen	0 ppm	100 ppm	2000 ppm	20000 ppm
Females on study (F_1 and F_0)	**F_0** **F_1**	29 28	29 30	30 28	30 30
No. dams pregnant	**F_0** **F_1**	29 24	24 28	29 26	27 30
Insemination index %	**F_0** **F_1**	100 100	100 100	100 96.4	100 96.4
Fertility index %	**F_0** **F_1**	96.7 76.6	83.3 93.1	96.7 88.8	90.00 96.4
Gestation index	**F_0** **F_1**	100 100	95.2 100	100 100	100 100
Duration of pregnancy	**F_0** **F_1**	22.4 22.4	22.2 22.2	22.2 22.2	22.0 22.0
No of pups (total)*	**F_1** **F_2**	229 232	216 298	306 278	231 314
No of pups dead	**F_1** **F_2**	3 1	5 16	9 1	5 1
Mean implantation sites per dam	**F_1** **F_2**	11.26 10.66	11 12.95	11.75 11.0	10.04 11.06
Live birth index	**F_1** **F_2**	98.9 99.5	97.8 95	96.6 97.7	97.8 99.7
% males/%females	**F_1** **F_2**	49/51 50.2/49.8	49/51 52.4/47/6	53/47 52.5/47/5	53/47 53.8/46.2
Mean Litter size	**F_1** **F_2**	10.00 9.6	10.28 10.32	10.37 10.69	10.04 10.46
Mean litter weight	**F_1: d0** **d28**	59.40 446.71	60.19 531.4++	60.50 462.39	57.66 342.18+

	$F_{2:}$ d0 **d28**	60.70 328.37		62.09 291.61		65.60 342.70		61.32 283.79	
Viability index (PN 4)	**F_1** **F_2**	92.8 89.9		93.4 89.8		96.1 88.6		94.4 86.4	
Mean pup weight during lactation	**F_1** 0 4 14 28 **F_2** **0** 4 14 28	♂ 6.36 9.50 28.55 72.94 6.54 9.40 26.42 73.17	♀ 5.89 9.35 26.89 66.10 6.15 8.83 26.42 73.17	♂ 6.22 9.98 29.91 74.57 6.23 8.63 28.26 75.44	♀ 5.98 9.85 29.16 68.99 5.88 8.56 26.88 67.44	♂ 6.33 9.50 28.93 72.25 6.44 8.95 25.58 69.09	♀ 5.99 9.14 28.41 68.40 6.08 8.54 26.88 67.82	♂ 5.96 8.80 27.17 64.68↓+ 6.25 8.94 25.94 66.59↓+	♀ 5.69 8.84 26.69 61.23↓ 5.91 8.31 23.86 59.41↓++
Lactation index (%)	**$F_{1:}$** d21 d28 **$F_{2:}$** d21 d28	84.4 84.4 58.2 58.2		95.4** 95.4** 55.9 55.9		86 86 75 75		66.5↓** 66.5↓** 56.4 56.4	
Mean relative liver weight **Weanlings (no statistical analysis)**	**$F_{2♂}$**	4442		4331		4565↑		5127↑↑	
	$F_{2♀}$	4278		4249		4522↑		4837↑↑	

U-test: + = 5%, ++ = 1%. * Fishers test = 1%, ** = 5%

Offspring

Mortalities/clinical signs: There were no mortalities and no clinical signs in pups up to weaning.

Body weights: In the F_1 generation, there was a statistically significant reduction in litter weight (23.5%) at weaning in the 20000 ppm dose group. In F_2 pups, litter weights were reduced by 13.5% compared to control values on day 28 (not statistically significant). Individual pup birth weights and body weight gains of F_1 pups during lactation were unaffected up to 2000 ppm. At 20000 ppm, lower pup body weights (male pups sometimes $p \leq 0.05$) were recorded during lactation. Individual birth weights of F_2 pups were unaffected up to 20000 ppm. Mean body weights of male and female F_2 pups during lactation were unaffected up to 2000 ppm, but significantly lower body weights were noted in both male pups ($p \leq 0.05$) and females pups ($p \leq 0.01$) in the 20000 ppm group at day 28.

Viability and lactation index: The mean viability indices of the treatment groups were comparable with those of the control. The lactation of F_1 pups was not affected up to the dose of 2000 ppm, but dams in the 20000 ppm group showed a significantly lower mean lactation index than controls. The viability (day 4 p.p.) of treated F_2 pups was comparable with controls. Up to 20000 ppm there was no dose-dependent reduction in the lactation indices calculated for the treatment groups. However, compared with the F_1 offspring, a relatively low lactation index was observed . A relatively high incidence of cannibalism occurred in all groups, when compared with F_1 pups. No explanation is given for this.

Offspring pathology: No treatment-related macroscopic alterations or skeletal deviations were observed in any of the F_1 or F_2 pups at any stage, up to levels of 20000 ppm. No gross pathological findings were made in either F_1 or F_2 weanlings at scheduled necropsy.

Organ weights: The mean weights of the brain, spleen, thymus, testes and ovaries showed no notable difference between the controls and treatment groups. The liver weights of weanlings showed no significant difference up to 2000 ppm. Male and female F_2 weanlings receiving 20000 ppm showed 13 to 15% higher relative liver weights compared to controls.

Developmental milestones: Maturation of external sexual organs in males and females was not influenced by treatment.

Conclusion

The parental LOAEL = 20000 ppm (2509 mg/kg bw/day), was based on reductions in body weight and increases in liver weights of both sexes at this dose level, with cytoplasmic changes in hepatocytes. Although these cytoplasmic changes were also observed at 2000 ppm, liver weights and other parameters were not affected at this dose level and therefore the effect was not regarded as adverse. The reproductive LOAEL = 20000 ppm (2509 mg/kg bw/day), was based on reduced body weight in F_1 and F_2 pups during lactation, reduced mean litter weight at weaning (day 28) in F_1 pups, an increase in relative liver weights in F_2 weanlings, and a reduced lactation index in F1 pups at 20000 ppm. Effects seen in pups of both generations during lactation were most likely to result from ingestion of test material in the chow during this period and not considered to reflect a toxic effect *via* lactation.

4.11.1.2 Human information

No data.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

Study 1: Developmental toxicity study in rats after oral administration. DAR/RAR B.6.6.2.1.

Iprovalicarb (95.8%) was suspended in 0.5% Tylose in demineralized water, and administered daily by gavage to groups of 28 (29 in the 100 mg/kg group) inseminated Wistar (strain Hsd/Cpb:WU) rats from day 6 to day 15 *post coitum* (p.c.) in doses of 0, 100, 300 or 1000 mg/kg bw/day. The animals of all groups received a uniform volume of 10 ml/kg bw. Animals were acclimatised for 7 days after arrival at the facility, prior to mating. At the time of mating, the males weighed > 300 g, and the females ranged in weights from 196 to 247 g on day 0 p.c..

General parameters investigated in the dams included general appearance and behaviour, feed and water consumption, the appearance of their excretory products, body weight development and mortality of the animals, as well as pathological findings. Caesarean sections were performed on the animals on gestation day 20. After weighing and opening the uterus, the numbers of live and dead foetuses, the number of (early/late) resorptions, the individual weight and appearance of the placentae, and the number of corpora lutea were recorded.

The foetuses were examined for gross abnormalities, sexed, and weighed. Approximately half of the foetuses were evaluated for visceral malformations and other deviations from normal. Findings in abdominal, pelvic, and thoracic organs during evisceration of the foetuses were

selected for skeletal evaluation. Staining of the skeletal system was carried out on the rest of the foetuses and was done using alizarin red S.

Dams were subjected to gross pathological evaluation at the time of caesarean section on day 20 p.c.

Results:

Mortality/Clinical signs: The dams were unaffected with respect to clinical signs and mortality up to and including the highest dose tested (1000 mg/kg). Food and water consumption was not adversely affected.

Body weights/pathology: Body weight gain of the dams was not affected at any dose tested when compared to control animals up to and including 1000 mg/kg and there were no treatment-related effects were noted at necropsy up to and including the highest dose.

Pregnancy endpoints: Fertility rates (% of inseminated animals with implantations) in the dose groups did not differ significantly from the control dams, and were also within the range of historical control data. The number of *corpora lutea*, pre-implantation losses and implantations was comparable in all treated groups, with the exception of the significantly lower number of *corpora lutea* in the 100 mg/kg group, not considered related to treatment (Table 40). Neither the weight of the placentae, the resorption rate, the mean number of foetuses, the sex ratio nor the foetal weight were affected by treatment with doses up to and including the highest dose (1000 mg/kg) (Table 40).

Foetal endpoints: A small number of statistically significant deviations with respect to stage of ossification and skeletal variation were observed. These were not dose-dependent in incident.

Neither the type nor the incidence rate of malformations were affected by the treatment at doses up to and including 1000 mg/kg b.w.. The number of abnormal foetuses at all dose levels was slightly lower when compared to the control group. One malformation of the spinal column was observed in the 100 mg/kg dose group, but this is not considered to be of toxicological significance due to the lack of dose-response. All other findings in the control and the treatment groups of this study were also observed at comparable incidence rates in control groups of this or of previous studies.

Table 51: Summary of reproductive endpoints

Dose (mg/kg bw/day)	**0**	**100**	**300**	**1000**
Inseminated animals	28	29	28	28
No. evaluated	28	29	28	28
Animals with implantations	23	20	24	23
Fertility rate (%)	82.1	69.0	85.7	82.1
Dams with viable foetuses	23	20	24	23
Gestation rate	100.0	100.0	100.0	100.0
Mean values per dam with viable foetuses				

Corpora lutea	15.3	13.5*	14.4	14.9
Pre-implantation loss	2.8	1.8	2.0	2.5
Implantations	12.5	11.8	12.5	12.4
Placental weight (g)	0.61	0.61	0.60	0.61
Number of foetuses	11.8	11.1	11.8	11.6
Resorptions	0.7	0.6	0.7	0.8
% males	49.5	47.6	51.0	53.9
Foetal weight (g)	3.72	3.70	3.72	3.78

* statistically significant difference from controls, < 0.05

Conclusions

There was no evidence of developmental toxicity potential of iprovalicarb observed in this study, at any dose level tested, up to and including 1000 mg/kg b.w./day.

Study 2: Developmental toxicity study in rabbits after oral administration. DAR/RAR B.6.6.2.2

Iprovalicarb (95.8%) was suspended in 0.5% Tylose in demineralized water, and administered orally daily by gavage to groups of 16 female inseminated Russian rabbits (strain CHBB:HM) from day 6 to day 18 *post coitum* (p.c.) in doses of 0, 100, 300 or 1000 mg/kg bw/day. The animals of all groups received a uniform volume of 10 ml/kg bw. Animals were acclimatised after arrival at the facility, before initiating treatment. At the time of mating, the males were mature to breed, and the mature females' body weights ranged from 2121 to 3062 g on day 0 p.c.

Evaluation of the general tolerance of the test compound by the pregnant females was based on general appearance and behaviour, feed and water intake, appearance of excretory products, body weight development and mortality of the animals, as well as gross pathological findings. Caesarian sections were performed on the animals on gestation day 29. After weighing and opening the uterus, the numbers of live and dead foetuses, the number of (early/late) resorptions, the individual weight and appearance of the placentae, and the number of corpora lutea were recorded.

After removal from the uterus, the foetuses were examined for gross abnormalities and their sex and body weight were determined. Approximately half of the foetuses were evaluated for visceral malformations and other deviations from normal. Findings in abdominal, pelvic, and thoracic organs during evisceration of the foetuses were selected for skeletal evaluation. Staining of the skeletal system was carried out on the rest of the foetuses and was done using alizarin red S.

Dams were subjected to gross pathological evaluation at the time of caesarian section on day 29 p.c.

Results

Mortality/Clinical signs: The dams were unaffected with respect to clinical signs and mortality up to and including the highest dose tested (1000 mg/kg). Food and water consumption was not adversely affected.

Body weights/pathology: Body weight gain of the dams was not affected at any dose tested when compared to control animals up to and including 1000 mg/kg and there were no treatment-related effects were noted at necropsy up to and including the highest dose.

Pregnancy endpoints: Fertility rates (% of inseminated animals with implantations) in the dose groups did not differ significantly from the control dams, and were also within the range of historical control data. The number of *corpora lutea*, pre-implantation losses and implantations was comparable in all treated groups and within historical control data ranges. Neither the weight of the placentae, the resorption rate, the mean number of foetuses, the sex ratio nor the foetal weight were affected by treatment with doses up to and including the highest dose (1000 mg/kg) (Table 41).

Foetal endpoints: Foetal ossification revealed some statistically significant events at the sternebrae, when calculated on both an individual and litter basis, indicating accelerated ossification in the treated groups compared to the control.

Table 52: Ossification status of the sternebrae

Dose level [mg/kg bw/day]	0	100	300	1000
Number of foetuses with …				
Sternebrae ossification, 5th				
- unossified [%]	39.5	20.0*	21.7*	10.5**
- incomplete ossification [%]	56.8	76.2*	68.9	86.3**
Number of litters with …				
Sternebrae ossification, 5th				
- unossified [%]	68.8	73.3	56.3	46.7
- incomplete ossification [%]	87.5	100.0	100.0	100.0

* = p<0.05 ** = p<0.01 (CHI2-Test and Fisher's Exact Test)

Table 53: historical control data: Skeletal findings (1991-1992)

Study number/year	T3039597 1991	T4040262 1991	T4040127 1991	T4040749 1992
Findings	**Number/%**	**Number/%**	**Number/%**	**Number/%**
Foetal incidence				
Sternum (incompletely ossified)				
-1st sternebrae	-	1/1/0%	-	-
-2nd sternabrae	-	1/1.0%	-	-
-5th sternabrae	77/81.1%	68/68.7%	62/68.9%	58/72.5%
(Unossified) 5th sternebrae	12/12.6%	23/23.2%	25/27/8%	15/18/8%
Litter incidence				
Sternum (incompletely ossified)				
-1st sternebrae	-	-	-	-
-2nd sternabrae	-	-	-	-
-5th sternabrae	14/100%	15/100%	14/100%	13/100%
(Unossified)				

5th sternebrae	8/57.1%	10/66.7%	8/57.1%	5/46.2

Historical control data (4 studies 1991-1992) were supplied for this strain of rabbit (CHBB:HM). The % (foetal) unossified 5th sternebrae was 12.6, 23.2, 27.8 and 18.8%. This puts the incidence in the control group for this study as high (39.5%)(well outside historical controls), the incidence in the low and intermediate levels within historical controls (20 and 21.7%) and the incidence in the high dose (10.5%) was slightly outside historical controls. The historical control range (4 studies) for incompletely ossified 5th sternebrae (% litter) is 81, 68.7, 68.9, and 72.5. The control group for this study is therefore outside (lower than) the historical data and only the high dose is slightly higher. When the data are considered on a *per* litter basis there is no statistically significant difference. The increased ossification seen in this study is not considered treatment-related and of questionable biological relevance in any case. No other locations were identified as having accelerated ossification.

Neither the incidence nor type of malformations was affected by the treatment up to and including the dose of 1000 mg/kg bw/day.

Conclusions

There was no evidence of developmental toxicity potential of iprovalicarb observed in this study, at any dose level tested, up to and including 1000 mg/kg b.w./day.

4.11.2.2 Human information

No data.

4.11.3 Other relevant information

No data.

4.11.4 Summary and discussion of reproductive toxicity

The reproductive toxicity of Iprovalicarb was investigated in a two-generation study in rats, and in developmental toxicity studies in rats and rabbits.

A concentration of 2000 ppm in the feed, over two generations, had no adverse effect on reproductive parameters. Parental animals of both generations showed signs of liver enzyme induction at 2000 ppm (cytoplasmic changes in hepatocytes which was not considered to be adverse). At the high dose level (20000 ppm), body weights were reduced and liver weights of both sexes were increased in the parental animals. At the highest dose also, pup body weights during the lactation phase were reduced in both the F_1 and F_2 generations, slightly reduced mean litter weight at birth and at weaning (day 28) in F_1 pups, an increase in relative liver weights in F_2 weanlings, and a reduced lactation index in F_1 pups were also recorded at the high dose level.

The administration of iprovalicarb does not have a direct effect on reproduction and clinical effects are seen only at the high dose. An LOAEL = 20000 ppm (2509 mg/kg bw/day) was established for both parental and reproductive toxicity.

In species tested, iprovalicarb up to and including the highest dose level (1000 mg/kg bw), was tolerated well without any signs of toxicity either in dams or on intrauterine development. The

maternal and developmental NOAEL/NOEL in both the rat and the rabbit is 1000 mg/kg bw, the highest dose tested.

In conclusion, iprovalicarb shows no evidence of either reprotoxic or embryotoxic potential in either species tested.

4.11.5 Comparison with criteria

Category 1A: Human evidence (epidemiological) is required for classification in this category, as there is no human evidence, iprovalicarb does not classify as Repr. 1A.

Category 1B and Category 2: A concentration of 2000 ppm (146.3 mg/kg bw/day) in the feed, over two generations, had no adverse effect on reproductive parameters. There was no evidence of a developmental toxicity potential. Neither the type nor the incidence rate of malformations were affected by the treatment at doses up to and including 1000 mg/kg b.w. Classification in Repr. 1B or 2 for fertility or development is not supported based on lack of effect. The administration of iprovalicarb does not have a direct effect on reproduction and clinical effects are seen only at the high dose.

4.11.6 Conclusions on classification and labelling

No classification.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS proposed no classification of iprovalicarb for reproductive toxicity on the basis of the following studies:

- One developmental toxicity study with Wistar rats where no effects were seen up to and including the limit dose of 1000 mg/kg bw/day.
- One developmental toxicity with Russian rabbits where no effects were seen up to and including the limit dose of 1000 mg/kg bw/day.
- One 2-generation reproduction toxicity study with Wistar rats, where the following effects were observed: reduced body weight in F1 (23.5%) and F2 (13.5%) pups during lactation, reduced (23%) mean litter weight at weaning in F1 pups, increase (15%) in relative liver weights in F2 weanlings, and reduced (21%) lactation index in F1 pups at doses well above 1000 mg/kg bw/day in both sexes.

Comments received during public consultation

One MSCA supported the DS's proposal for no classification of iprovalicarb as regards as reproductive toxicity.

Assessment and comparison with the classification criteria

Study 1: Two generation study in Wistar rats (DAR/RAR B.6.6.1)

Groups of 30 male and 30 female Wistar (strain ICO:WU (IOPS Cpb)) rats were administered iprovalicarb (99.2%) at dietary concentrations of 0, 100, 2000 or 20000 ppm over 2 generations in an OECD guideline compliant study. The test substance intake is summarised in Table 12.

Table 12: *Test substance intake of F0 and F1 parents*

F0 GENERATION				F1 GENERATION			
Dose (ppm)	Sex	Weeks	mg/kg bw/day	Dose (ppm)	Sex	Weeks	mg/kg bw/day
100	male	10	7.3	100	male	15	7.7
2000	male	10	146	2000	male	15	155
20000	male	10	1514	20000	male	15	1838
100	female	10	9.6	100	female	15	11
2000	female	10	190	2000	female	15	240
20000	female	10	2074	20000	female	15	2944

There was no evidence of treatment-related mortality in male or female F0 and F1 animals at levels of up to 20000 ppm and no test substance-related effects on the appearance or behaviour.

The body weights of the male and female F0 and F1 animals receiving 100 or 2000 ppm did not differ significantly from those of controls. In the 20000 ppm group, males of the F0 generation exhibited slightly reduced body weights (maximally 6%, $p < 0.05$) from week 13 onwards, while the F1 generation males had significantly depressed body weights (approx. 10% in week 13). In 20000 ppm females of the F0 generation, a slight to moderate body weight depression was detected during premating (< 5%), and lactation (up to 7%) period. In the F1 females, the body weight reduction reached statistical significance only during week 13 and on lactation day 14.

Effects on parental generations

Pathology

No significant gross pathological or histopathological findings were observed at necropsy of male or female F0 and F1 animals at doses of up to 20000 ppm. No noticeable discrepancies between implantation sites and number delivered pups occurred in any of the treatment groups. Post implantation losses were comparable in treated and untreated rats.

Organ weights

In F0 parents, the weights of the liver and testes were comparable with those of the controls up to 2000 ppm. In the 20000 ppm group, higher relative liver weights were recorded in males (14%) and in females (22.2%). In the 20000 ppm males, relative testes weights were elevated by 10%.

In F1 parents, the absolute (males only) and relative (males and females) liver weights were significantly elevated. Relative weights were raised by 11.4% and 28.3% in males

and females respectively. Other organ weights (including kidney weights determined in F1 rats) showed no deviation between treated and untreated groups that were dose-dependent.

Histopathology

In both generations, there were no treatment-related alterations of liver morphology in the 100 ppm group. However, in both the 2000 and 20000 ppm groups, a majority of rats exhibited minimal to slight cytoplasmic changes in the hepatocytes.

Spermatological evaluation

In F0 males, a slight reduction in initial (at 1 minute) sperm motility was detected in the 20000 ppm group. However, all but one (out of a total of 15 males) was found to be fertile. There was no dose-related effect on sperm motility. Some sperm abnormalities (head-tail break, head-tail separation or sharps in the tail) were found more frequently at 20000 ppm. However, this is mainly due to a high head-tail separation incidence in one particular animal. In F1 males, no effect was detected on any sperm parameters at 20000 ppm. Therefore, males of the 100 and 2000 ppm were not examined in this respect.

Oestrus cycle length

No significant change in either generation.

Reproductive parameters

Fertility indices were unaffected by treatment. There were no effects on litter size, pup weights, sex ratio or on viability of these pups up to PND 4. Mating performance of the F0 or the F1 animals was not affected by the treatment at levels of up to 20000 ppm.

Offspring

Body weights

In the F1 generation, there was a statistically significant reduction in litter weight (23.5%) at weaning in the 20000 ppm dose group. In F2 pups, litter weights were reduced by 13.5% compared to control values on day 28 (not statistically significant). Individual pup birth weights and body weight gains of F1 pups during lactation were unaffected up to 2000 ppm. At 20000 ppm, lower pup body weights (male pups, sometimes $p \leq 0.05$) were recorded during lactation. Individual birth weights of F2 pups were unaffected up to 20000 ppm. Mean body weights of male and female F2 pups during lactation were unaffected up to 2000 ppm, but significantly lower body weights were noted in both male pups ($p \leq 0.05$) and females pups ($p \leq 0.01$) in the 20000 ppm group at day 28.

Viability and lactation index

The mean viability indices of the treatment groups were comparable with those of the control. The lactation of F1 pups was not affected up to the dose of 2000 ppm, but dams in the 20000 ppm group showed a significantly lower mean lactation index than controls. The viability (PND 4) of treated F2 pups was comparable with controls. Up to 20000 ppm there was no dose-dependent reduction in the lactation indices calculated for the treatment groups. However, compared with the F1 offspring, a relatively low lactation

index was observed. A relatively high incidence of cannibalism occurred in all groups, when compared with F1 pups. No explanation was given.

Offspring pathology

No treatment-related macroscopic alterations or skeletal deviations were observed in any of the F1 or F2 pups at any stage, up to levels of 20000 ppm. No gross pathological findings were observed in either F1 or F2 weanlings at scheduled necropsy.

Organ weights

The mean weights of the brain, spleen, thymus, testes and ovaries showed no notable difference between the controls and treatment groups. The liver weights of weanlings showed no significant difference up to 2000 ppm. Male and female F2 weanlings receiving 20000 ppm showed 13 to 15% higher relative liver weights compared to controls.

Developmental milestones

Maturation of external sexual organs in males and females was not influenced by treatment.

Conclusion

The parental LOAEL = 20000 ppm (2509 mg/kg bw/day), was based on reductions in body weight and increases in liver weights of both sexes at this dose level, with cytoplasmic changes in hepatocytes. Although these cytoplasmic changes were also observed at 2000 ppm, liver weights and other parameters were not affected at this dose level and therefore the effect was not regarded as adverse.

The reproductive LOAEL = 20000 ppm (2509 mg/kg bw/day) was based on reduced body weight in F1 and F2 pups during lactation, reduced mean litter weight at weaning (day 28) in F1 pups, an increase in relative liver weights in F2 weanlings, and a reduced lactation index in F1 pups at 20000 ppm. Effects seen in pups of both generations during lactation were most likely to result from ingestion of test material in the feed during this period and not considered to reflect a toxic effect via lactation.

Study 2: Developmental toxicity study in rats after oral administration (DAR/RAR B.6.6.2.1)

Iprovalicarb (95.8%) was suspended in 0.5% Tylose in demineralized water, and administered daily by gavage to groups of 28 (29 in the 100 mg/kg group) inseminated Wistar rats from day 6 to day 15 post coitum in doses of 0, 100, 300 or 1000 mg/kg bw/day.

Mortality/Clinical signs

The dams were unaffected with respect to clinical signs and mortality up to and including the highest dose tested (1000 mg/kg bw/day).

Body weights/pathology

Body weight gain of the dams was not affected at any dose tested when compared to control animals up to and including 1000 mg/kg bw/day and no treatment-related effects were noted at necropsy up to and including the highest dose.

Pregnancy endpoints

Fertility rates (% of inseminated animals with implantations) in the dose groups did not differ significantly from the control dams, and were also within the range of historical control data. The number of corpora lutea, pre-implantation losses and implantations was comparable in all treated groups, with the exception of the significantly lower (88% of control) number of corpora lutea in the 100 mg/kg bw/day group, not considered related to treatment. Neither the weight of the placentae, the resorption rate, the mean number of foetuses, the sex ratio nor the foetal weight were affected by treatment with doses up to and including the highest dose (1000 mg/kg bw/day).

Foetal endpoints

A small number of statistically significant deviations with respect to stage of ossification and skeletal variation were observed. These were not dose-dependent.

Neither the type nor the incidence rate of malformations was affected by the treatment at doses up to and including 1000 mg/kg bw/day. The number of abnormal foetuses at all dose levels was slightly lower when compared to the control group. One malformation of the spinal column was observed in the 100 mg/kg bw/day, but this is not considered to be of toxicological significance due to the lack of dose-response. All other findings in the control and the treatment groups of this study were also observed at comparable incidence rates in control groups of this or of previous studies.

Conclusions

There was no evidence of developmental toxicity potential of iprovalicarb observed in this study, at any dose level tested, up to and including 1000 mg/kg bw/day.

Study 3: Developmental toxicity study in rabbits after oral administration (DAR/RAR B.6.6.2.2)

Iprovalicarb (95.8%) was suspended in 0.5% Tylose in demineralized water, and administered orally daily by gavage to groups of 16 female inseminated Russian rabbits from day 6 to day 18 post coitum in doses of 0, 100, 300 or 1000 mg/kg bw/day.

Mortality/Clinical signs

The dams were unaffected with respect to clinical signs and mortality up to and including the highest dose tested (1000 mg/kg bw/day).

Body weights/pathology

Body weight gain of the dams was not affected at any dose tested when compared to control animals up to and including 1000 mg/kg bw/day and no treatment-related effects were noted at necropsy up to and including the highest dose.

Pregnancy endpoints

Fertility rates (% of inseminated animals with implantations) in the dose groups did not differ significantly from the control dams, and were also within the range of HCD. The number of corpora lutea, pre-implantation losses and implantations was comparable in all treated groups and within historical control data ranges. Neither the weight of the

placentae, the resorption rate, the mean number of foetuses, the sex ratio nor the foetal weight were affected by treatment with doses up to and including the highest dose (1000 mg/kg bw/day).

Foetal endpoints

Foetal ossification revealed some statistically significant events at the sternebrae, when calculated on both an individual and litter basis, indicating accelerated ossification in the treated groups compared to the control. Table 13 shows the incidence per foetuses and per litter of the alterations in ossification of sternebrae.

Table 13: *Ossification status of the sternebrae. Historical control data contains references from 4 studies: T3039597 (1991), T4040262 (1991), T4040127 (1991) and T4040749 (1992).*

	mg/kg bw/day				Historical control data (%)			
	0	100	300	1000	T3039597 (1991)	T4040262 (1991)	T4040127 (1991)	T4040749 (1992)
Number of foetuses with…								
Unossified (%)	39.5	20.0*	21.7*	10.5**	12.6	23.2	27.8	18.8
Incomplete ossification (%)	56.8	76.2*	68.9	86.3**	81.1	68.7	68.9	72.5
Number of litters with…								
Unossified (%)	68.8	73.3	56.3	46.7	57.1	66.7	57.1	46.2
Incomplete ossification (%)	87.5	100	100	100	100	100	100	100

** and ** = statistically significant regarding the control for p < 0.05 and p < 0.01, respectively.*

Historical control data (4 studies 1991-1992) were supplied for this strain of rabbit. The percentages of foetal unossified 5th sternebrae were 12.6, 23.2, 27.8 and 18.8%. This puts the incidence in the control group for this study (39.5%) well outside historical controls, the incidence in the low and intermediate levels within historical controls (20 and 21.7%) and the incidence in the high dose (10.5%) slightly outside historical controls. This same historical control data for incompletely ossified 5th sternebrae (% litter) are 81.1, 68.7, 68.9, and 72.5%. The control group for this study was therefore lower than the historical data and only the high dose is slightly higher.

When the data are considered on a per litter basis there is no statistically significant difference. The increased ossification seen in this study is not considered treatment-related and of questionable biological relevance in any case. No other locations were identified as having accelerated ossification.

Neither the incidence nor type of malformations was affected by the treatment up to and including the dose of 1000 mg/kg bw/day.

Conclusions

There was no evidence of developmental toxicity potential of iprovalicarb observed in this study, at any dose level tested, up to and including 1000 mg/kg bw/day.

Comparison with criteria

Slight alterations in the following parameters were reported in a 2-generation reproduction study in rats at doses always well above 1000 mg/kg bw/day for all generations; i) pup body weights during the lactation in both the F1 and F2 generations; ii) mean litter weight at birth and at weaning (day 28) in F1 pups; iii) relative liver weights in F2 weanlings; and, iv) a reduced lactation index in F1 pups. The severity of

these effects were not considered sufficient for supporting a classification, especially considering that the dose was higher than the dose of 1000 mg/kg bw/day typically considered as limit dose.

No developmental impairments were found in foetuses of rabbits and rats exposed to 1000 mg/kg bw/day.

Thus, RAC supports the DS's proposal for **no classification of iprovalicarb regarding reproductive toxicity.**

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

Table 54: Summary table of relevant neurotoxicity studies

Method	Results	Remarks	Reference
Acute 28 day oral gavage study in rats. Wistar strain Hsd Cpb:WU. AS: 97.7% pure 0, 176, 806, and 1680 mg/kg bw	No effects seen up to and including the limit dose of 1680 mg/kg bw.	GLP, guideline = yes. NOAEL (neurotoxicity) = 1680 mg/kg bw/day.	Dreist, M., and Popp, A., 1997 DAR/RAR B.6.7.1.1
Subchronic 90-day oral dietary study in rats. Wistar strain Hsd Cpb:WU. AS: 97.7% pure M: 0, 86, 342 and 1434 mg/kg bw F: 0, 131, 476 and 2314 mg/kg bw/day	There were no treatment-related clinical signs and no deaths in either sex at any dietary exposure level. Body weight gain was reduced in males by 10% at the two highest dose levels. Increased food consumption (high-dose males +10% and females +12%). A marginal decrease in measures of motor activity in high-dose females at week 8.	GLP, guideline = yes. NOAEL (neurotoxicity) = 1434 mg/kg bw/day (males).	Dreist, M., and Popp, A., 1997 DAR/RAR B.6.7.1.2

In an acute neurotoxicity screening study, none of the investigated parameters was affected by treatment with iprovalicarb. The highest dose (2000 mg/kg bw) was tolerated without any neurotoxic effects in either sex.

In the subchronic neurotoxicity study, evidence of slight, dose-related, non-specific general toxicity was noted. The only neurobehavioural effect observed was a (reversible), marginal decrease in the motor activity measurements in females of the highest dose group. However, this finding was regarded to be secondary to non-specific toxicity and thus not considered to represent a neurotoxic effect *per se*. In addition, there were no histopathological correlatives

observed in the skeletal muscle or in the nervous tissues. The highest dose tested (20000 ppm) represents the NOAEL for neurotoxicity.

In conclusion, iprovalicarb does not have a neurotoxic potential, after either acute or subchronic administration.

4.12.1.2 Immunotoxicity

No data.

4.12.1.3 Specific investigations: other studies

No data.

4.12.1.4 Human information

No data.

4.12.2 Summary and discussion

No effects from the neurotoxicity studies to support a proposal for classification.

4.12.3 Comparison with criteria

None required.

4.12.4 Conclusions on classification and labelling

Not required.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The data presented in this section is reproduced directly from the Draft Assessment Report (RAR) for Iprovalicarb in summary form or as robust study summeries, as appropriate. The Draft Assessment Report (RAR) for Iprovalicarb is prepared in accordance with Reg. (EC) No. 1107/2009 concerning the placing of Plant Protection Products on the market

5.1 Degradation

Table 55: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis			
EPA: Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, § 161 1, Hydrolysis Studies, October 18, 1982	Iprovalicarb was stable at pH 5, 7 and 9 and no hydrolysis products were observed. Iprovalicarb accounted for ≈ 100% of the label recovered.	No further studies are neccessary	RAR: Henneböle, 1996b. B8.4.1
Photolysis in water			
Phototransformation of Chemicals in Water, Part A: Direct Phototransformation, UBA, Berlin, FRG; December 1992	The UV-VIS absorption data in the environmentally relevant pH range (buffers at pH 5, 7 and 9) showed that Iprovalicarb (SZX 0722) in aqueous solutions does not absorb any light at wavelengths above 281 nm.	Direct photodegradation in aqueous solution will not contribute to the overall elimination of Iprovalicarb in the environment.	RAR: Hellpointner, 1994. B.8.4.2

5.1.1 Stability

Route and rate of degradation in aquatic systems (chemical and photochemical degradation).

Hydrolytic Degradation: (Henneböle, 1996b). The hydrolysis of Iprovalicarb was studied in 0.01 M buffer solutions adjusted to pH 5, 7 and 9. The test solutions were prepared with radiolabelled parent compound ([phenyl-UL-14C]) at a concentration of about 1.44 mg/L. The solutions were incubated for a maximum period of 30 days under sterile conditions in the dark at 25°C, and the sampling intervals were 0, 3, 7, 14, 20, 24 and 30 days post-treatment.

The sterility of the test solutions was checked in subsamples over the incubation period and no contamination was observed. The pH of the test solutions remained constant during the study period. The total recovery of radioactivity was on average 100.3% (± 1.8%), 100.3 (± 1.3%) and 99.6 (± 1.7%) of the amount applied during the incubation period of 30 days at pH 5, 7 and 9.

Iprovalicarb was stable at pH 5, 7 and 9 and no formation of hydrolysis products was observed. In TLC analysis, Iprovalicarb accounted for approximately 100% of the radioactivity recovered from the solutions at termination of the experiment. Considering the hydrolytic stability determined under environmental pH and temperature conditions, it is not expected that hydrolytic processes will contribute to the degradation of iprovalicarb in the aqueous environment.

Photochemical Degradation: (Hellpointner, 1994). To assess the direct photodegradation of organic chemicals in water under environmental conditions, computer models (Zepp and Cline 1977; Frank and Klopffer 1985) were developed which required the quantum yield of direct photodegradation in water as an input parameter for the determination of the so called 'environmental half-life'. The determination of the quantum yield of the direct photodegradation in water is only meaningful if the molar extinction coefficient of the test compound in water above 295 nm is greater than 10 L $mole^{-1}$ cm^{-1} in which case the determination is necessary for an assessment of the environmental behaviour. Iprovalicarb was dissolved in various buffer solutions at pH 5, 7 and 9 and the UV-VIS adsorption spectrum was first assessed over the wavelength 190 to 750 nm.

The only chromophor present in the Iprovalicarb molecule is the substituted aromatic ring. The results indicated that Iprovalicarb in aqueous solutions did not absorb any light at wavelengths above 281 nm. Determination of the quantum yield in this case was meaningless. Based on this result, it is expected that direct photodegradation in the aqueous solution will not contribute to the overall elimination of Iprovalicarb in the aquatic environment.

5.1.2 Biodegradation

A study on the Ready Biodegradability of Iprovalicarb was not performed. Iprovalicarb is considered not to be 'readily biodegradable'. A water/sediment study (Henneböle, J. (1997c)) in two water/sediment systems under aerobic conditions indicated no significant mineralization until well after 30 days (4 – 7% at day 30, 8 – 27% at day 60). Samples were incubated in the dark under aerobic conditions at 20-21°C for a maximum period of 100 days. Sampling dates were 0, 1, 3, 7, 14, 30, 60 and 100 days after application. There was still 44-81% of the applied radioactivity remaining in the water layers at 30 days with the sediment layer accounting for 12 – 45% at this time point. There is no evidence to suggest that Iprovalicarb can be degraded in the aquatic environment to a level > 70% within a 28 day period.

5.1.2.1 Biodegradation estimation

No data.

5.1.2.2 Screening tests

No data.

5.1.2.3 Simulation tests

No data.

5.1.3 Summary and discussion of degradation

Iprovalicarb is not considered to be 'readily biodegradable'. It is both hydrolytically and photolytically stable at environmentally relevant pH values.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

The adsorption/desorption behaviour of Iprovalicarb investigated in a batch equilibrium study was evaluated during the Annex I Inclusion using the phenyl-labelled parent compound, submitted within the EU Basic Dossier 1998. The applicant also submitted a new soil adsorption/desorption study performed according to the Brazilian Guidelines which accepted for inclusion in the RAR. The study was included since it enlarged the data set on the leaching behaviour of Iprovalicarb. For a better overview of the results of both adsorption/desorption studies a short summary is given at the end of this section.

Study 1: The adsorption/desorption measurements were carried out on five different soils using four different concentrations of Iprovalicarb (RAR B8.2: Henneböle, J. (1996a): Adsorption/Desorption of SZX 0722 on soils). The adsorption constants Kd calculated from the Freundlich isotherms for the five soils range from 0.25 to 1.27 ml/g. When recalculating the Kd values with the organic C content of the soils, Koc values of 61 to 131 ml/g were obtained. The calculated desorption Kd values range from 1.35 to 3.61 ml/g, and corresponding Koc values from 176 to 674 ml/g. On the basis of these findings, Iprovalicarb can be classified as being a "mobile substance" to a "substance of low mobility" (Briggs 1973, Verdam et al 1988) with a Koc range of 61-131 for the test soils.The results are shown in Table 22 below.

Study 2: This study was included in the RAR in order to enlarge the data set on adsorption (RAR B8.1.2.1: Bonetti, R. 2000, Soil adsorption/desorption of SZX 0722 TÈCNICO). The adsorption/desorption measurements were carried out on three different soils using four different concentrations of Iprovalicarb. The adsorption constants Kf were calculated by means of the Freundlich adsorption isotherm and ranged from 0.77 to 4.64 mL/g. These values were normalised to the organic carbon content and corresponded to Koc values between 44 and 221 mL/g with an arithmetic mean of 132 mL/g. While there were some shortcomings with the study the results were considered reliable for combination with the results from Study 1. These combined results are shown in Table 22.

Kfoc (ads) values were also determined for the single diastereomers of iprovalicarb using a HPLC method. These were identical for the two diastereomers, therefore it was concluded that the adsorption behaviour on soil is identical for both diastereomers.

Table 56: Summary of relevant information on adsorption/desorption

Soil	Adsorption				Desorption			
	1/n	K_f [mL/g]	K_{oc} [mL/g]	r^2	1/n	K_f [mL/g]	K_{oc} [mL/g]	r^2
Borstel, Germany[a)]	0.9150	0.8360	121	0.9999	0.9346	1.8881	274	0.9998
Howe, USA[a)]	0.8595	1.0037	90	0.9988	0.9123	2.4939	223	0.9997
Stanley, USA[a)]	0.8410	1.2682	131	1.0000	0.8139	3.6051	372	0.9990
Napa, USA[a)]	0.8821	0.6003	61	0.9999	0.7809	1.7453	176	0.9941
GH, Brazil[b)]	0.93	4.64	131	0.9994	1.04	1.43	40	0.9964

LR, Brazil[b)]	0.83	0.76	44	0.9988	1.06	0.76	44	0.9997
LE, Brazil[b)]	0.85	0.77	221	0.9979	1.03	0.78	224	0.9999
arith. Mean	*0.8725*		*114*		*0.9388*		*193*	

a) Study 1, Henneboele (1998). Note: the Vero Beach soil from this study omitted due to o.c. < 0.2%
b) Study 2, Bonetti (2000)

5.2.2 Volatilisation

The vapour pressure of 7.9 × 10-8 Pa at 20oC (isomer mix.) and 2.1 x 10-7 Pa at 25oC (isomer mix.), and the molecular weight (~320.4) allow that it will not readily volatilize into the atmosphere at ambient temperature and pressure.

5.2.3 Distribution modelling

Not relevant for this dossier.

5.3 Aquatic Bioaccumulation

Table 57: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
OECD 305 E, EPA 72-6, EPA 165-4	10	Very low BCF-whole fish	RAR: Dorgerloh, B9.2.2.4.1

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Based on the on the Log K_{ow} [Log K_{ow} = 3.18 (Diastereomer A and Log K_{ow} = 3.2 (Diastereomer B)] there is a possibility for bioaccumulation for the substance SZX0722. A theoretical calculation of the potential Log K_{ow} for the metabolites M10 and M15 indicated values of 2.03 and 1.98, respectively, suggesting low potential for bioaccumulation.

5.3.1.2 Measured bioaccumulation data

A bioconcentration test for Iprovalicarb was conducted in fish. Fifty six young bluegill *Lepomis macrochirus* were exposed per test unit. A dosing system was used to maintain mean water concentrations (nominal) of 20 µg and 200 µg [14C]-SZX 0722/l (Iprovalicarb) for a 28-day exposure period. After exposure the test fish were placed in clean water for 14 days, to determine the depuration of [^{14}C]-SZX 0722. Stock solution of test substance was dissolved in acetone. The test aquarium was replenished with aerated water about 6 times in a 24 hour period. The control aquarium also received an amount of acetone equivalent to the positive exposure group. The initial loading of the aquaria was 2.5 g fish/l and 0.42 g fish/l/day.

Fish were observed initially and every 24 hours for mortality and adverse behaviour. Following the uptake phase of 28 days the aquaria were cleaned and fish exposed to uncontaminated diluent water for 14 days. Fish were sampled (4/aquarium) at regular intervals over the uptake

and depuration phases and dissected into fillet, (edible parts) and viscera. At similar intervals water samples were taken for analytical determination of test substance concentration.

Radio analyses (14C CO2) of edible and non-edible portions of individual fish were performed at defined time points. At the same time radioactivity in water samples was measured.

A non-linear two-compartment kinetic model (BIOFAC 2) was used to analyse the uptake/depuration data. Both control analysis of the stock solutions and radioactivity determinations of the test medium showed exposure concentrations were maintained over the test period. The mean exposure concentrations during the uptake phase were 19.2 (± 0.5) µg/l and 206.2 (± 5.4) µg/l which compares well with expected nominal values.

The mean tissue residues (mg/kg fresh weight) at steady state were 0.07, 0.43, 0.21 mg/kg for edible/ non edible and whole fish for 20 µg as/l and 0.66, 3.40, 1.72 mg/kg for edible, non-edible and whole fish for 200 µg as/l respectively.

All BCF values may be overestimated in this study because all calculations refer to the total amount of radioactivity (sum of radiolabelled parent, metabolites and mineralisation products).

Iprovalicarb is accumulated and excreted very rapidly by Bluegill Sunfish with a mean bioconcentration factor of 10 for whole fish.

5.3.2 Summary and discussion of aquatic bioaccumulation

With an experimentally derived BCF of 10, Iprovalicarb is considered not likely to bioaccumulate.

5.4 Aquatic toxicity

A brief summary is presented below of the aquatic toxicity studies listed in the RAR for the three trophic levels: fish, aquatic invertebrates and algae/aquatic plants.

Table 58: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
EG C.1, EPA 72-1, OECD 203	LC_{50} >22.7 mg/L	Static *Oncorhynchus mykiss*	RAR: Dorgerloh, B9.2.1.1
EG C.1, EPA 72-1, OECD 203	$LC_{50} \geq$ 20.7 mg/L	Static *Lepomis macrochirus*	RAR: Dorgerloh, B9.2.1.2
OECD 204, OECD draft TG\94.214, ISO 10229	NOEC mg/L ≥ 9.89	Semi-static: *Oncorhynchus mykiss*	RAR: Dorgerloh, B9.2.2.1
OPPTS number 850.1400; ASTM StanRARd E1241-88a	ELS NOEC = 5.0 mg/L	Flow-thorugh: *Oncorhynchus mykiss*	RAR: Drottar and Krueger, B9.2.2.2
OECD 202, EPA 72-2	$EC_{50} \geq$ 19.8 mg/L	Static: *Daphnia magna*	RAR: Heimbach, B9.2.4.1

OECD 202, EPA 72-4, EEC XI/681/86	NOEC = 1.89 mg/L	Static renewal: *Daphnia magna*	RAR: Heimbach, B9.2.5.1
OECD 201, EEC Directive 79/831/E, EPA 738-R-94-035, ISO 8692	E_rC_{50} > 10 mg/L $NOE_rC \geq$ 10 mg/L	Static: *Selenastrum capricornnutum*	RAR: Anderson, B9.2.6.1
OECD Guideline 218	$EC_{15emerg}$ > 128mg/kg NOEC = 125 mg/kg	Spiked sediment: *Chironomus riparius*	RAR: Bruns, B9.2.8.1

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Study 1: Young Rainbow Trout (*Oncorhynchus mykiss)* were exposed to a single nominal concentration of Iprovalicarb (SZX 0722) for 96 h in a static limit test. The concentration tested was 22.7 mg/L. The test substance was dissolved with dimethylformamide. During the test, fish were examined at four hours and then daily for mortality and symptoms of intoxication. The active substance was very stable during the test with measured values between 22.5 and 23.0 mg/L at day 4. No toxic symptoms were noted during the test, therefore the LC_{50} > 22.7 mg/L (nominal) and the NOEC ≥ 22.7 mg/L (nominal).

Study 2: Young Bluegill Sunfish (*Lepomis macrochirus*) were exposed to a single nominal concentration of Iprovalicarb (SZX 0722) for 96 h in a static limit test. The concentration tested was 20.7 mg/L. The test substance was dissolved with dimethylformamide. During the test, fish were examined at four hours and then daily for mortality and symptoms of intoxication. The active substance was very stable during the test with measured values between 22.5 and 23.0 mg/L at day 4. No toxic symptoms were noted during the test, therefore the LC_{50} > 20.7 mg/L (nominal) and the NOEC ≥ 20.7 mg/L (nominal).

5.4.1.2 Long-term toxicity to fish

Study 1: Juvenile Rainbow Trout (*Oncorhynchus mykiss)* were exposed to five concentrations of Iprovalicarb (SZX 0722) for 28 days in a flow-through test system. The test concentrations were of 0, 0-solvent, 0.63, 1.25, 2.5, 5 and 10 mg/L. The test substance was dissolved with dimethylformamide. During the test, fish were monitored daily for mortalities and three times per week for toxic symptoms. All fish were weighed at day 0, 14 and 28. The active substance was very stable during the test. Purity testing on the substance found it to contain 98.9% pure a.i. The EC_{20} and LOEC values were determined as >9.89 mg a.i. /L (nominal), which was the highest concentration tested. The NOEC was therefore determined to be >9.89 mg a.i./L (nominal).

Study 2: Early Life Stage Toxicity Test: Newly fertilised Rainbow Trout (*Oncorhynchus mykiss)* were exposed to five concentrations of Iprovalicarb (SZX 0722) for 88 days. The nominal concentrations (mean measured) were negative control, solvent control, 0.63 (0.65), 1.3 (1.3), 2.5 (2.6), 5.0 (5.0) and 10 (9.1) mg a.s./L; mean measured concentrations represent 103, 100, 104, 100 and 91% of nominal. The NOEC was determined as 5.0 mg/L (mean measured).

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Study 1: Young *Daphnia magna* were exposed to Iprovalicarb (SZX 0722) for 48h in a semi-static test system. The mean measured test concentrations were: 0, 6.02, 10.5 and 19.8 mg a.i./L. The highest test concentration was the limit of solubility. The test substance was dissolved with dimethylformamide. The active substance was very stable during the test. All daphnids were unfed and evaluated daily for mortality, immobility and sublethal effects. Test concentrations were measured at the beginning and end of the test period. The EC_{50} was the limit of solubility. The EC_{50} > 19.8 mg/L (mean measured) the highest dose tested (NOEC ≥ 19.8 mg/L (mean measured).

5.4.2.2 Long-term toxicity to aquatic invertebrates

Study 1: Young *Daphnia magna* were exposed to Iprovalicarb (SZX 0722) for 21 days under static renewal conditions to mean measured concentrations of: 0.36, 1.04, 1.89, 3.41, 5.81 and 10.6 mg/L. Fifteen animals were used to record mortality and an additional 10 animals per concentration for other parental and reproduction parameters. The test substance was dissolved with dimethylformamide. Test solutions were replaced three times a week. The active substance was very stable during the test. At 3.41 to 10.6 mg/L mean body length was significantly reduced. Some other effects noted were not considered treatment related due to a lack of dose response. Iprovalicarb has a moderate chronic toxicity to *Daphnia magna* with a paternal NOEC of 1.89 mg/L and a reproduction NOEC of 5.81 mg/L. Therefore the overall NOEC was considered to be 1.89 mg/L (mean measured).

5.4.3 Algae and aquatic plants

Study 1: *Selenastrum capricornutum* (strain 61.81) was exposed under static conditions (shake cultures) for 96 h to concentrations (nominal) of Iprovalicarb of 1.0, 1.80, 3.20, 5.60 and 10.0 mg/L. The test substance was dissolved with dimethylformamide. No abnormalities, e.g. morphological changes or effects on the growth rate were observed. Up to its limit of water solubility, Iprovalicarb is not toxic to *Selenastrum capricornutum*. Therefore, ErC_{50} > 10 mg/L and NOErC ≥ 10 mg/L.

5.4.4 Other aquatic organisms (including sediment)

Study 1: Larvae of *Chironomus riparius* in their first instar were exposed in a static test system for 28 days to Iprovalicarb at initial nominal concentrations of: 7.81, 15.6, 31.3, 62.5, 125 and 250 mg/ kg dw sed (dry weight sediment) of a water-sediment system. The concentrations of iprovalicarb were analysed in the freshly prepared spiked sediments of all test concentrations and the controls on day -2. The concentrations were analysed on days 0, 7 and day 28 (after insertion of the larvae) in the overlying water, the pore water and the sediment. Accompanying chemical analyses were performed using additionally separate test vessels for all test concentrations and controls. Test conditions met all validity criteria, given by the guideline. The EC_{15} for iprovalicarb in the 28 day study with *Chironomus riparius* was determined to be 128 mg/kg dw sed. for emergence ratio and > 250 mg /kg dw sed. for development rate. The NOEC was determined to be 125 mg/kg dw sed. for emergence ratio and ≥ 250 mg/kg dw sed. These concentrations are all based on initial nominal concentrations.

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

A complete set of studies for the three required aquatic trophic levels covering acute and chronic toxicity were submitted for the evaluation of the substance iprovalicarb. All of the studies found aquatic endpoints to be > 1 mg/L. Therefore there is no classification under CLP for aquatic toxicity. Evaluation of potential bioaccumulation indicated a BCF of 10 and the Log k_{ow} = 3.18 (Diastereomer A) and Log k_{ow} = 3.2 (Diastereomer B) indicating no basis of classification under CLH criteria.

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

CLP

Iprovalicarb does not classify under CLP environmental criteria.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Degradation

The DS proposed to consider iprovalicarb as not rapidly degradable for classification purposes. The basis for this proposal is that iprovalicarb is both hydrolytically stable (Henneböle, 1996b) and photolytically stable (Hellpointner, 1994) at environmentally relevant pH values. A study on the ready biodegradability of iprovalicarb is not available. A water/sediment study (Henneböle, 1997c) in two water/sediment systems under aerobic conditions indicated no significant mineralization until well after 30 days (4 – 7% at day 30, 8 – 27% at day 60). Both water/sediment systems showed a strong mineralisation of iprovalicarb with a maximum of 79.9% applied radioactivity (AR) in Anglersee and 65.3% AR in Hoenniger Weiher water/sediment system at study termination. The residues for Anglersee test systems were 0.1% AR at day 0. They increased to a maximum of 25.1% AR at day 60 and decreased again to 15.2% AR at study termination. For Hoenniger Weiher test systems, the residues were 0.1% AR at day 0 and increased to a maximum of 23.3% AR at study termination (see B.8.2.2.2 - Water/Sediment studies). In the total system, the SFO $DegT_{50}$ were in the range of 19.92 to 58.67 days.

Aquatic Bioaccumulation

The DS proposed to not consider iprovalicarb as being bioaccumulative in the aquatic environment for classification purposes. The basis for this proposal is an experimentally (OECD TG 305) derived BCF of 10 (*Lepomis macrochirus,* Dorgerloh, 1997). The octanol/water partition coefficients ($LogK_{OW}$) have been determined as 3.18 (Diastereomer A) and 3.20 (Diastereomer B).

Acute and Chronic Aquatic Toxicity

The DS proposed to not classify iprovalicarb as Acute nor Chronic toxic for the aquatic environment. The basis for this proposal is a complete set of studies for the three required

aquatic trophic levels as well as for the sediment aquatic organism *Chironomus riparius* covering acute and chronic toxicity. All of the studies found aquatic endpoints to be > 1 mg/L.

Table 14: *summary of the acute and chronic aquatic toxicity studies*

Method	Results	Remarks	Reference
EG C.1, EPA 72-1, OECD TG 203 GLP (yes) Iprovalicarb: 97.1%	LC_{50} > 22.7 mg/L (mean measured)	Static*:* *Oncorhynchus mykiss*	RAR: B9.2.1.1 Anon., 1995a
EG C.1, EPA 72-1, OECD TG 203 GLP (yes) Iprovalicarb: 97.1%	LC_{50} > 20.7 mg/L (mean measured)	Static*:* *Lepomis macrochirus*	RAR: B9.2.1.2 Anon., 1995b
OECD TG 204, OECD draft TG\94.214, ISO 10229[#] GLP (yes) Iprovalicarb: 98.9%	NOEC mg/L ≥ 9.89 mg/L (mean measured. All measured concentrations ranged from 84 to 99% of nominal)	Semi-static: *Oncorhynchus mykiss*	RAR: B9.2.2.1 Anon., 1997
OPPTS number 850.1400; ASTM StanRARd E1241-88a GLP (yes) Iprovalicarb: 97.6%	ELS NOEC = 5.0 mg/L (mean measured)	Flow-through: *Oncorhynchus mykiss*	RAR: B9.2.2.2 Anon., 2000
OECD TG 202, EPA 72-2 GLP (yes) Iprovalicarb: 97.5%	EC_{50} > 19.8 mg/L (mean measured)	Static: *Daphnia magna*	RAR: B9.2.4.1 Heimbach, 1996
OECD TG 202, EPA 72-4, EEC XI/681/86 GLP (yes) Iprovalicarb: 97.0%	$NOEC_{parental}$ = 1.89 mg/L (mean measured) $NOEC_{repr}$ = 5.81 mg/L (mean measured)	Static renewal: *Daphnia magna*	RAR: B9.2.5.1 Heimbach, 1996
OECD TG 201, EEC Directive 79/831/E, EPA 738-R-94-035, ISO 8692 GLP (yes) Iprovalicarb: 97.0%	E_rC_{50} > 10 mg/L NOE_rC ≥ 10 mg/L (Quantitative analysis at day 0 showed 103% of nominal concentrations. All results are expressed in nominal terms).	Static: *Selenastrum capricornutum*	RAR: B9.2.6.1 Anderson, 1996
OECD TG 218[#], GLP (yes) Iprovalicarb: 97.5%	$EC_{15emerg}$ > 128 mg/kg NOEC = 125 mg/kg (nominal).	Spiked sediment: *Chironomus riparius*	RAR: B9.2.8.1 Bruns, 2010

[#] the OECD TG 204 test with *Oncorhynchus mykiss* and the OECD TG 218 test with *Chironomus riparius* were not used for hazard classification

.

Comments received during public consultation

Three MSCAs commented on the proposals for environmental classification, all agreeing with the conclusion that no classification is warranted for the environment.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the proposal of the DS to consider iprovalicarb as not rapidly degradable for classification purposes.

Aquatic Bioaccumulation

Based on a BCF of 10 and a $LogK_{OW}$ of 3.2, RAC agrees with the proposal of the dossier submitter to not consider iprovalicarb as being bioaccumulative in the aquatic environment for classification purposes.

Acute Aquatic Toxicity

For all trophic levels, the acute toxicity studies resulted in $L(E)C_{50}$ above 1 mg/L, therefore RAC agrees with the DS' proposal to not classify iprovalicarb as acute toxic for the aquatic environment.

Chronic Aquatic Toxicity

Iprovalicarb is not considered rapidly degradable, nor bioaccumulative and the results from the chronic studies did not show chronic toxicity within the guidance values for classification. Therefore, RAC agrees with the DS' proposal to not classify iprovalicarb as chronic toxic for the aquatic environment.

Overall, RAC supports the DS's proposal for **no classification of iprovalicarb as hazardous to the aquatic environment.**

6 OTHER INFORMATION

None.

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8 ANNEXES

Documentation supporting this CLH report is attached in section 13 of the CLH dossier in the form of the relevant parts of the RAR (2016).