Committee for Risk Assessment

RAC

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

Adopted

30 November 2018

30 November 2018

CLH-O-0000001412-86-237/F

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

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

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

EC Number: **-**

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

The proposal was submitted by **Ireland** and received by RAC on **19 October 2017.**

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

Ireland has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **16 January 2018**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **19 March 2018**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Miguel A. Sogorb**

Co-Rapporteur, appointed by RAC: **Michael Neumann**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **30 November 2018** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

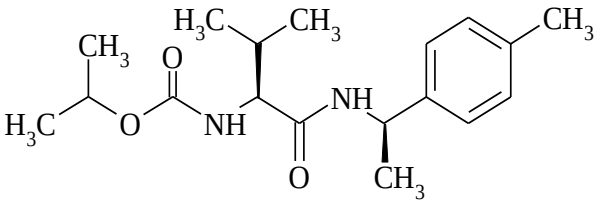
	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	iprovalicarb (ISO); isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate	-	140923-17-7	Carc. 2	H351	GHS08 Wng	H351			
RAC opinion	TBD	iprovalicarb (ISO); isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate	-	140923-17-7	Carc. 2	H351	GHS08 Wng	H351			
Resulting Annex VI entry if agreed by COM	TBD	iprovalicarb (ISO); isopropyl [(2S)-3-methyl-1-{[1-(4-methylphenyl)ethyl]amino}-1-oxobutan-2-yl]carbamate	-	140923-17-7	Carc. 2	H351	GHS08 Wng	H351			

GROUNDS FOR ADOPTION OF THE OPINION

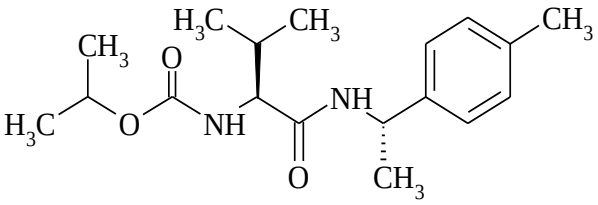
RAC general comment

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*)



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.

HUMAN HEALTH HAZARD EVALUATION

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 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 No gross pathological changes LD_{50} > 5000 mg/kg bw for both sexes	Bomann, 1993; 22108 RAR B.6.2.1.2
OECD TG 402, GLP Dermal	98.4% iprovalicarb 5000 mg/kg bw	No mortalities No clinical signs or skin changes	Bomann, 1993 Study No. T4041135

Bor:WISW rats Male/female 5 animals/sex Vehicle: 2% Cremophor in physiological saline	After 24 hours of occlusive exposure the treated skin area was cleaned with soap and water	No gross pathological changes LD_{50} > 5000 mg/kg bw for both sexes	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 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	Bomann, 1993 Study No. T3041134 RAR B.6.2.7

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.

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**.

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 2 females, 1 male Vehicle: polyethylene glycol	500 mg 98.4% iprovalicarb moistened with vehicle Semi-occlusive dressing Exposure period: 4 hours and afterwards the exposed skin areas were carefully washed with water.	No sign of any irritation at any time point (24, 48 and 72 hours post-application) No signs of any systemic toxicity in any of the treated animals Non-irritant	Krötlinger, 1992 Study No. T2041197 RAR 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.**

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**.

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 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.	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 preparations without iprovalicarb	No signs of irritation after challenge	Kolb, 1992 Study No. T1041178

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.**

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 (see background document) summarises the main findings in the repeated dose toxicity studies with iprovalicarb in animals.

Data presented in the table 5 (see background document) 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 (see background document) 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, background document); 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.**

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) OECD TG 473, GLP	Chinese hamster V79 cells	0,120,180 mg/ml of 96.86% iprovalicab	With S9: negative Without S9: negative	Positive controls worked well with and without S9 mix	Herbold, 2001; 31333 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	Brendler-Schwaab, 1996 RAR B.6.4.1.7

				Dose levels at or below 150 µg/ml were non-toxic. Positive controls worked well with and without S9 mix	

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.**

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 (see background document) 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[1]				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[2]				Concluded a somewhat decreasing trend but without statistical significance				
HED 0050652[3]				Male: 0.7 (0.0-4.0) Female: 0.6 (0.0-2)	Male: 0.1 (0.0-2.0) Female: 0			

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

[2] 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.

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

Carlus *et al.* 2013[1]	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[2]	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 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.

[1] 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.

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

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 (see background document) 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 the 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, such 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 a 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, for the above stated reasons RAC considers that the evidence of carcinogenicity is limited and therefore supports the DS's proposal for **classifying iprovalicarb as carcinogenic category 2 (H351; suspected of causing cancer).**

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.**

ENVIRONMENTAL HAZARD EVALUATION

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.**

ANNEXES:

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

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