Bayer CropScience

[redacted]

Document Title

Tier 2 Summary of KIIA 5 Toxicological and Toxicokinetic Studies on the Active Substance for Iprovalicarb (SZX 0722)

(Specification No.: 102000006810-05)

Substance(s)

IPROVALICARB (ANNEX I RENEWAL)

Data Requirements

EU Commission Regulation No. 1141/2010 on the renewal of the inclusion of AIR2 active substances in conjunction with Directive 91/414/EEC and Regulation EC/1107/2009

Annex IIA Section 3, Point 5 Document M

According to OECD format guidance for industry data submissions on plant protection products and their active substances

Date

2013-10-04

Author(s)

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Bayer CropScience

OWNERSHIP STATEMENT

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KIIA 5 - Toxicological and Toxicokinetic Studies on the Active Substance

Comments with respect to the Annex I renewal process

This delta dossier contains only summaries of studies, which were not available at the time of the first Annex I inclusion of iprovalicarb and were therefore not evaluated during the first EU review of this compound. The summaries on the different toxicological endpoints from the baseline dossier were supplemented and adapted with the new information. In order to facilitate discrimination between new information and original paragraphs, the new information is written in bold italic letters. All other studies, which were already submitted by Bayer for the first Annex I inclusion, are contained in the Monograph and in the baseline dossier provided by Bayer CropScience.

A synonymous name for iprovalicarb used at several locations in this delta dossier is SZX0722.

Addendum October 2013: A brief summary on the "Expert panel consensus report in assessing the weight of evidence of iprovalicarb carcinogenicity in rats and mice" (M-422060-01-1) has been included in this summary, after the submission of this statement had been requested by RMS Ireland in the course of their evaluation. A position paper prepared by [redacted] on the biological relevance of the replication fraction (RF) is also included (M-461421-02-1). Please find these additional summaries in chapter "KIIA 5.5.4 - Mechanism of action and supporting data", for easy reference printed in blue type.

KIIA 5.1 - Absorption, distribution, excretion and metabolism in mammals

KIIA 5.1.1 - Toxicokinetic studies - Single dose, oral route, in rats

A rat metabolism (ADME) study conducted with [phenyl-UL-^{14}C]iprovalicarb was submitted with the original EU dossier. Since the metabolisation is well understood, no additional rat metabolism studies have been carried out.

Following oral administration (low dose), iprovalicarb was rapidly and almost completely absorbed as indicated by a bile fistulation study. It was widely distributed in the body with the highest residues in the liver. However, there is no evidence for accumulation in any tissue based on studies on plasma kinetics and autoradiography. Excretion of absorbed iprovalicarb was rapid and complete; 98 % of the oral dose was excreted within 48 hours after dosing.

The metabolism of iprovalicarb in the rat is extensive with a metabolisation rate of >90 % of the dose and 12 metabolites have been identified in the excreta. Consequently, unchanged iprovalicarb accounted for <10 % of the (low) dose (and 16-21 % of the high dose). The metabolic pathway proceeds mainly via oxidation of the 4-methyl phenyl group to iprovalicarb carboxylic acid, partly followed by conjugation with glycine and taurine. Minor metabolites are formed by hydroxylation of the phenyl ring and cleavage of the molecule. The metabolism study was conducted in high quality. The total recovery of radioactivity ranged from 90 to 107 % of the administered dose. The rate of identification accounted for 80 – 90 % of the dose independent of sex and dose level.

Please refer to point IIA 5.1 (EU point 5.1) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

Report: [redacted];[redacted];1997;M-000019-01
Title: [Phenyl-UL-14C]SZX 0722: Investigation of the biokinetic behaviour and the metabolism in the rat
Report No: PF4263
Document No: M-000019-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

KIIA 5.1.2 - Toxicokinetic studies - Second single dose, oral route, in rats

Report: [redacted];[redacted];1997;M-000019-01
Title: [Phenyl-UL-14C]SZX 0722: Investigation of the biokinetic behaviour and the metabolism in the rat
Report No: PF4263
Document No: M-000019-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

KIIA 5.1.3 - Toxicokinetic studies - Repeated dose, oral route, in rats

Report: [redacted];[redacted];[redacted];1997;M-001057-01
Title: [14C] SZX 0722: Investigation of the biokinetic behaviour and the metabolism in the rat following subchronic feeding
Report No: PF4323
Document No: M-001057-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

KIIA 5.2 - Acute toxicity

Summary of acute toxicity, irritation and sensitisation studies

Table 5.2-1: Summary of acute toxicity, irritation and sensitisation studies

Species	Sex	Vehicle	NSD/NSC [mg/kgbw]	LLD/LLC [mg/kg bw]	LD/LC_{50} [mg/kg bw]	Reference
Acute oral toxicity						
rat	male female	water/ cremophor 2%v/v	5000 5000	> 5000 > 5000	> 5000 > 5000	[redacted], 1993; 22110
mouse	male female	water/ cremophor 2%v/v	5000 5000	> 5000 > 5000	> 5000 > 5000	[redacted], 1993; 22108
Acute percutaneous toxicity						
rat	male female	water/ cremophor 2%v/v	5000 5000	> 5000 > 5000	> 5000 > 5000	[redacted], 1993; 22107
Acute inhalation toxicity						
rat	male female		4977 4977	> 4977 > 4977	> 4977 > 4977	Pauluhn, 1993; 22368

NSD/NSC: no-symptoms dose/concentration; LLC/LLD: lowest lethal dose/concentration

Species	Sex		Vehicle	Result	Reference
Irritation					
rabbit	Femal male	skin eye	moistened polyethylene glycol none	not irritating not irritating	[redacted], 1992; 21713
Sensitisation					
guinea pig	female	maximisation test	Cremophor® EL (2%v/v) / phys.saline solution	not sensitising	[redacted], 1992; 21938
Acute intra peritoneal rat	Male female		water/ cremophor 2%v/v	LD > 500 mg/kg	[redacted], 1993; 22109

Iprovalicarb has no significant acute toxicity after oral, dermal and inhalatory administration. The compound is neither a skin nor an eye irritant. No skin-sensitising potential was found in the maximisation test.

Please refer to point IIA 5.2 (EU point 5.2) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.2.1 - Acute oral toxicity

Report:	[redacted];[redacted];1993;M-000355-01
Title:	SZX 0722 - Investigations of acute oral toxicity in rats
Report No:	22110
Document No:	M-000355-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1993;M-000368-01
Title:	SZX 0722 - Study for acute oral toxicity to mice
Report No:	22108
Document No:	M-000368-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.2 - Acute percutaneous toxicity

Report:	[redacted];[redacted];1993;M-000374-01
Title:	SZX 0722 - Study for acute dermal toxicity in rats
Report No:	22107
Document No:	M-000374-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.3 - Acute inhalation toxicity

Report:	[redacted];[redacted];1993;M-000337-01
Title:	SZX 0722 - Acute inhalation toxicity study on rats in compliance with OECD No. 403
Report No:	22368
Document No:	M-000337-01-2
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.4 - Skin irritation

Report:	[redacted];[redacted];1992;M-000383-01
Title:	SZX 0722 - Study for skin and eye irritation/corrosion in rabbits
Report No:	21713
Document No:	M-000383-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.5 - Eye Irritation

Report:	[redacted];[redacted];1992;M-000383-01
Title:	SZX 0722 - Study for skin and eye irritation/corrosion in rabbits
Report No:	21713
Document No:	M-000383-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.6 - Skin sensitization

Report:	[redacted];[redacted];1992;M-000377-01
Title:	SZX 0722 - Study for skin-sensitizing effects in guinea pigs (Magnusson-Kligman maximization test)
Report No:	21938
Document No:	M-000377-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.2.7 - Potentiation/interactions of multiple active substances or products

KIIA 5.2.8 - Acute intraperitonial toxicity

Report:	[redacted];[redacted];1993;M-000361-01
Title:	SZX 0722 - Study for acute intraperitoneal toxicity in rats
Report No:	22109
Document No:	M-000361-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3 - Short-term toxicity

Summary of short-term toxicity studies

Table 5.3-1: Summary of short-term toxicity studies

Type of study	Species	Dose range tested (ppm)	NOAEL (equal to mg/kg bw)	Reference
oral, 4 weeks, gavage	rat	0,2000,6000,20000	2000 (196)	[redacted], 1995; 24489
oral, 13 weeks, feed	rat	0,1250,5000,20000	5000 (373)	[redacted], 1996; 24766
oral, 13 weeks, feed	mouse	0,280,1400,7000,14000	1400 (325)	[redacted], 1996; 25296
oral, 4 weeks, feed	dog	0,100,1000,10000,50000	100 (3.0)	[redacted], 1993; MTD0299
oral, 4 weeks, feed, supplementary	dog	0,10,20,40,80	20 (0.77)	[redacted], 1997; 26810
oral, 13 weeks, feed	dog	0,250,2500,50000	< 250 (9.1)	[redacted], 1995; 24337
oral, 52 weeks, feed	dog	0,80,800,8000	80 (2.6)	[redacted] et al., 1997; 26454
dermal, 4 weeks	rabbit	0-1000 kg bw/day	>1000 mg/kg bw/day	[redacted] et al., 1995; 24215
inhalation, 5 days	rat	0,20.6,102.9,504.4 mg/m3	>504.4 mg/m3	[redacted], 1993; 22369

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. The lowest short term NOAEL was established in female dogs following a 52-week treatment period, on the basis of which a sub-chronic NOAEL of 2.7 mg/kg/d was proposed. No NOAEL was observed in male dogs from this 52-week chronic toxicity study.

Please refer to point IIA 5.3 (EU point 5.3) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.3.1 - Oral 28-day toxicity

Report:	[redacted];[redacted];[redacted];1995;M-000448-02
Title:	SZX 0722 - Subacute oral toxicity study in Wistar rats (Twenty-eight day feeding study)
Report No:	24489
Document No:	M-000448-02-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];[redacted];[redacted];[redacted];1993;M-000390-01
Title:	Safety evaluation of SZX 0722: Four-week dietary toxicity study in dogs
Report No:	R5987
Document No:	M-000390-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**Yes**

Report:	[redacted];[redacted];1997;M-000578-01
Title:	**SZX 0722: Subacute toxicity study in Beagle dogs (additional)**
Report No:	**26810**
Document No:	**M-000578-01-1**
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3.2 - Oral 90-day toxicity (rodents)

Report:	[redacted];[redacted];[redacted];[redacted];1996;M-000440-01
Title:	SZX 0722 - Subchronic toxicological investigations in Wistar rats (Administration in feed over 13 weeks followed by a 4-week recovery period)
Report No:	24766
Document No:	M-000440-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];[redacted];1996;M-000436-01
Title:	SZX 0722 - Dose-range-finding study in B6C3F1 mice (Administration in food over 13 weeks)
Report No:	25396
Document No:	M-000436-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3.3 - Oral 90-day toxicity (dog)

Report:	[redacted];[redacted];[redacted];1995;M-000453-02; Amended: 1998-03-17
Title:	SZX 0722 - Subchronic toxicity in dogs (13-week study by oral administration)
Report No:	24337
Document No:	M-000453-02-1
Guidelines:	**Deviation not specified**

GLP/GEP: **yes**

KIIA 5.3.4 - Oral 1 year toxicity (dog)

Report:	[redacted];[redacted];[redacted];1997;M-000572-02 -Amended: 1998-02-18
Title:	SZX 0722 - Chronic toxicity study in beagle dogs (53 week feeding study)
Report No:	26454
Document No:	M-000572-02-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3.5 - 28-day inhalation toxicity (rodents)

Report:	[redacted];[redacted];1993;M-000384-01
Title:	SZX 0722 - Range-finding subacute inhalation toxicity study on rats (exposure for five 6-hour periods)
Report No:	22369
Document No:	M-000384-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3.6 - 90-day inhalation toxicity (rodents)

KIIA 5.3.7 - Percutaneous 28-day toxicity (rodents)

Report:	[redacted];[redacted];1993;M-000277-01
Title:	SZX 0722 - Subacute dermal toxicity study on the rabbit
Report No:	24215
Document No:	M-000277-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.3.8 - Percutaneous 90-day toxicity (rodents)

Based on the results of the acute and 28-day percutaneous toxicity study with iprovalicarb, a subchronic study was not triggered.

KIIA 5.4 – Genotoxicity

Summary of genotoxicity testing

Table 5.4-1: Summary of genotoxicity testing

Test system	Test object	Concentration	Purity (%)	Result	Reference / report no.
Salmonella microsome test	S.typhimurium TA98, TA100, TA1535, TA1537	8-5000 µg/plate	98.1	negative	[redacted] 1994, 22842
Salmonella microsome test	S. typhimurium TA102	16-5000 µg/plate	96.8	negative	[redacted], 2001; 31334
Salmonella typhimurium and E. coli reverse mutation assay	***S.typhimurium TA98, TA100, TA1535, TA1537, E.coli WP2/uvrA***	***3-5000 µg/plate***	***95.7***	***negative***	[redacted], ***2012; 1466200***
HGPRT-Test	Chinese hamster lung cells (V79)	7.8-250 µg/mL 12.5-125 µg/ml	98.1	negative	[redacted], 1995; 23858
Cytogenetic study	Chinese hamster ovary cells*	6,30,150 µg/mL	98.7	negative	[redacted], 1995; 24403
Cytogenetic study- 18 hours treatment without metabolic activation	Chinese hamster V79 cells	60,120,180 mg/ml	96.8	negative	[redacted], 2001; 31333
Unscheduled DNA synthesis test	rat primary hepatocytes	50-500 µg/mL	98.1-99.4	negative	[redacted], 1996; 24963
Micronucleus test	NMRI mouse bone marrow cells	2000 mg/kg bw i.p.	96.7	negative	[redacted], 1995; 24016
32P-postlabelling assay**	Uterus and urinary bladder of rats	20000 and 10000 ppm in the feed	96.4	negative	[redacted], 1998; 27184

Iprovalicarb was negative in a complete battery of *in vitro* genotoxicity tests with or without metabolic activation. Results of *in vivo* tests such as mouse micronucleus and ^{32}P postlabeling assay on rat uterus and urinary bladder were negative as well.

In addition, a reverse mutation assay on S. typhimurium on the strain TA 102 and a chromosome aberration assay on Chinese V79 cells have been performed to comply with current standard regulatory guidelines. A third Ames test (M-428023-01-1) has been conducted in 2012 to comply with the new specification of the compound. Again this assay showed a negative response.

Please refer to point IIA 5.4 (EU point 5.4) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.4.1 - In vitro genotoxicity

Report:	[redacted];1994;M-000330-01
Title:	SZX 0722 - Salmonella/microsome test
Report No:	22842
Document No:	M-000330-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	[redacted];[redacted];1995;M-000316-01
Title:	SZX 0722 Mutagenicity study for the detection of induced forward mutations in the V79-HPRT assay in vitro
Report No:	23858
Document No:	M-000316-01-1
Guidelines:	Deviation not specified
GLP/GEP:	Yes
Report:	[redacted];[redacted];1995;M-000519-01
Title:	SZX 0722 - In vitro mammalian chromosome aberration test with chinese hamster ovary (CHO) cells
Report No:	24403
Document No:	M-000519-01-1
Guidelines:	Deviation not specified
GLP/GEP:	Yes
Report:	[redacted];[redacted];1996;M-000507-01
Title:	SZX 0722 - Test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro
Report No:	24963
Document No:	M-000507-01-1
Guidelines:	Deviation not specified
GLP/GEP:	yes
Report:	***[redacted];2004;M-071967-01***
Title:	***SZX 0722 - Salmonella/microsome test***
Report No:	***37331***
Document No:	***M-071967-01-1***
Guidelines:	***Deviation not specified***
GLP/GEP:	***Yes***

Executive summary:

In this in vitro assessment of the mutagenic potential of iprovalicarb (Batch number 898222005, 96.8% purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 102 were exposed to SZX0722 up to 5000 µg/plate, diluted in dimethyl sulfoxide (DMSO). Triplicate plates were used in both the presence and absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter.

Doses up to and including 1581 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. Substance precipitation occurred at the dose of 1581 µg per plate and above. Therefore, the test was no longer interpretable at 5000 µg per plate. Evidence of mutagenic activity of SZX0722 was not seen. No biologically

relevant increase in the mutant count, in compari[redacted]to negative controls was observed. All the positive control compounds produced expected increases in the number of revertant colonies, thereby demonstrating the sensitivity of the assay and the efficacy of the S9 mix.

Therefore, SZX0722 was non-mutagenic with or without S9 mix for Salmonella typhimurium TA 102 in the plate incorporation of the Salmonella/microsome test.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test Material: ***SZX0722***
- ***Description:*** ***fine white powder***
- ***Lot/Batch:*** ***898222005***
- ***Purity:*** ***96.8%***
- ***CAS*** ***140923-17-7***
- ***Stability of test compound:*** ***Stable at room temperature***

2. Control materials: Negative: Culture medium
Solvent: DMSO
Positive: Cumene hydroperoxide (Sigma) for TA 102 without S9 2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix.

3. Test organisms:
- ***Species: Salmonella typhimurium LT2 mutants***
- ***Strain: Histidine-auxotrophic strains TA 102***
- ***Source: Strains obtained from [redacted] in 1997 and stored in the laboratory***

4. Test compound concentrations:
- ***Plate incorporation assay: with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate***

5. Metabolic activation: The S9 fraction was isolated from the livers of Aroclor 1254 induced male Sprague Dawley rats.

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed between August 8 to August 16, 2001 at Bayer HealthCare AG (PH-PD P Health Care Toxicology).
The Salmonella/microsome test is a screening method which detects point mutations caused by chemical agents in vitro. Auxotrophic mutants of Salmonella typhimurium are used to demonstrate this effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.
1. Plate incorporation assay:
SZX0722 or the positive control material was dissolved in 0.1 mL of DMSO. DMSO (0.1 mL) containing SZX0722 or positive controls were added to glass vessels with 0.1 mL of bacterial cultures grown overnight, 0.5 mL of S9 mix or buffer and 2 mL of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional doses. If less than three doses were used for assessment, at least two repeats were performed.
2. Acceptance criteria:

The negative controls had to be within the expected range. The positive controls had to show sufficient effects. Title determinations had to demonstrate sufficient bacterial density in the suspension. The title was determined by the total bacterial counts taken on two plates for each concentration studied with S9 mix.

4. Assessment criteria

A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 102 and increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. RESULTS AND DISCUSSION

The colony number of each plate and mean values are listed for each dose in Tables 5.4.1.5-1 and 5.4.1.5-2. As may be seen, there was no indication of a bacteriotoxic effect of SZX0722 at doses of up to and including 1581 ug per plate. The total bacteria counts consistently produced results comparable to the negative controls, or differed only insignificantly. No inhibition of growth was noted as well. At 1581 ug per plate, the substance started to precipitate, so that for 5000 ug per plate no further evaluation was possible. TA 102 showed in the plate incorporation test no dose-related or biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9 mix (Table 5.4.1.5-1) and was confirmed by the results of the preincubation trials (Table 5.4.1.5-2).The positive controls cumene hydroperoxide and 2-aminoanthracene increased mutant counts to well over those of the negative controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

Table 5.4.1.5-1: Mean mutant colony counts

	Strain TA 102			
	Plate incorporation		Preincubation	
µg/plate	-S9	+S9	-S9	+S9
0	201	215	198	232
16	208	207	215	228
50	218	188	205	223
158	207	219	231	266
500	196	219	212	285
1581	236	231	206	253
5000	-	-	-	-
Cumene	397		379	
2-AA		453		500

III. CONCLUSION

In the absence of any increase in mutant counts, there was no indication of any mutagenic effect of SZX0722 in this test. Iprovalicarb was therefore considered to be non-mutagenic.

Report:	[redacted]***;2012;M-428023-01***
Title:	***Salmonella typhimurium and Escherichia coli reverse mutation assay with iprovalicarb***
Report No:	***1466200***
Document No:	***M-428023-01-1***
Guidelines:	***OECD 471 (1997), EEC Commission Directive 2000/32/EC Method B13/14 (2000), EPA 40 CFR part 160 OPPTS 870.5100 (August 1998) MAFF 12 Nousan No8628 (December 06, 2000)***
GLP/GEP:	***yes***

Executive summary:

In this in vitro assessment of the mutagenic potential of iprovalicarb (Batch number EDMZ019701, 95.7% purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and the Escherichia coli strain WP2 uvrA were exposed to iprovalicarb up to 5000 µg/plate, diluted in dimethyl sulfoxide (DMSO). For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of a phenobarbital/β-naphthoflavone-induced rat liver metabolic activation system (S9 mix). DMSO was also used as a negative control. Specific positive controls were used for each strain. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter.No increase in revertant colony numbers of any of the five tester strains was observed at any dose level neither in the presence or the absence of metabolic activation.
In conclusion, under the experimental conditions reported, iprovalicarb did not induce gene mutations by base pair changes or frameshifts in the genome of the strains used. Therefore, iprovalicarb is considered to be non-mutagenic in this Salmonella typhimurium and Escherichia coli reverse mutation assay.

I. MATERIAL AND METHODS

A. MATERIALS:

1. Test Material: ***iprovalicarb***
Lot/Batch: ***EDMZ019701***
Purity: ***95.7%***
CAS ***494793-67-3***
Stability of test compound: ***Stable at room temperature***

2. Control materials: ***Negative: Culture medium***
Solvent: DMSO
Positive: Sodium azide (Serva) for TA 1535 and TA 100 at 10 µg/plate, 4-Nitro-1,2-phenylene diamine (Merck-Schuchardt) for TA1537 at 50 µg/plate and TA 98 at 10 µg/plate, methyl methane sulfonate, MMS (Sigma Aldrich) for WP2uvrA at 3.0 µL/plate, 2-Aminoanthracene (Aldrich) for the activating effect of the S9 mix in all strains at 2.5 µg/plate.

3. Test organisms:
Species: Salmonella typhimurium LT2 mutants and Escherichia coli
Strain: Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and tryptophan-independent strain WP2 uvrA
Source: Strains obtained from [redacted] Germany)

4. Test compound concentrations:

Plate incorporation assay:	*For all strains with or without S9 mix: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
Pre-incubation assay:	*For all strains with or without S9 mix: 10, 33, 100, 333, 1000, 2500 and 5000 µg/plate*
5. Metabolic activation:	*The S9 fraction was isolated from the livers of Phenobarbital/β-Naphthoflavone induced male Sprague Dawley rats.*

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed between February 16 to February 28, 2012, at [redacted] ([redacted], Germany).

1. Plate incorporation assay:

Iprovalicarb or the positive control material was dissolved in 0.1 mL of DMSO. DMSO (0.1 mL) containing iprovalicarb or positive controls were added to glass vessels with 0.1 mL of bacterial cultures grown overnight, 0.5 mL of S9 mix or buffer and 2 mL of soft agar. The mixture was placed in a waterbath at 45°C for 30 seconds, shaken and overlaid onto Petri dishes containing solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were scored using an automated colony counter. Three plates were used, both with and without S9 mix, for each strain and dose. The doses for the first trial were routinely determined on the basis of a standard protocol with a maximum dose of 5000 µg/plate and at least 5 additional doses. If less than three doses were used for assessment, at least two repeats were performed.

2. Pre-incubation assay:

An independent repeat test was performed as pre-incubation of the previously described mixture in a water bath at 37°C for 60 minutes. At the end of the preincubation period, 2 mL of molten soft agar were added to the tubes, the content mixed and plated onto Petri dishes with solid agar. After 48 hours of incubation at 37°C, the numbers of revertant colonies were also scored using an automated colony counter.

3. Acceptance criteria:

The negative controls had to be within the expected range. The positive controls had to show sufficient effects.

4. Assessment criteria

A test item is considered as a mutagen if a biologically relevant increase in the number of revertants exceeding the threshold of twice (strains TA98, TA 100 and WP2uvrA) or trice (strains TA 1535 and TA 1537) the colony count of the corresponding solvent control is observed. A dose dependent increase is considered biologically relevant if the threshold is exceeded at more than one concentration.

II. RESULTS AND DISCUSSION

No toxic effects were observed in the test groups with or without metabolic activation.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with iprovalicarb at any dose level. This applied both to the tests with and without S9 mix (table 5.4.1.3-1) and was confirmed by the results of the pre-incubation trials (Table 5.4.1.3-2).

The positive controls caused a significant increase in the number of revertant colonies compared to the controls demonstrating the sensitivity of the system.

Table 5.4.1.3-1: Mean mutant colony counts (1st experiment)

Substance/concentration (µg/plate)	*TA 1535*	*TA 100*	*TA 1537*	*TA 98*	*WP2uvrA*
Without S9 mix					
iprovalicarb 0 (DMSO)	*22*	*101*	*15*	*39*	*55*
3	*24*	*96*	*16*	*35*	*47*
10	*23*	*113*	*15*	*40*	*41*
33	*28*	*109*	*16*	*26*	*47*
100	*24*	*126*	*17*	*33*	*53*
333	*26*	*112*	*12*	*35*	*53*
1000	*26 p*	*109 p*	*15 p*	*37 p*	*55 p*
2500	*26 p,m*	*110 p,m*	*15 p,m*	*37 p,m*	*63 p,m*
5000	*23 p,m*	*129 p*	*17 p,m*	*33 p,m*	*55 p,m*
Na-azide 10	*2047*	*2402*	*-*	*-*	*-*
4-NOPD 10	*-*	*-*	*-*	*329*	*-*
4-NOPD 50	*-*	*-*	*83*	*-*	*-*
MMS 3 µl	*-*	*-*	*-*	*-*	*780*
With S9 mix					
iprovalicarb 0 (DMSO)	*28*	*135*	*20*	*52*	*66*
3	*31*	*127*	*18*	*46*	*62*
10	*36*	*121*	*23*	*45*	*61*
33	*31*	*136*	*21*	*51*	*70*
100	*35*	*134*	*27*	*52*	*65*
333	*29*	*132*	*23*	*50*	*69*
1000	*30 p*	*146 p*	*28 p*	*43 p*	*56 p*
2500	*27 p,m*	*133 p,m*	*22 p,m*	*48 p,m*	*59 p,m*
5000	*28 p, m*	*136 p,m*	*20 p,m*	*51 p,m*	*58 p,m*
2-AA 2.5	*449*	*2891*	*301*	*2384*	*-*
2-AA 10	*-*	*-*	*-*	*-*	*410*

P: precipitate; m: manudl count

Table 5.4.1.3-2: Mean mutant colony counts (2nd experiment)

Substance/concentration (µg/plate)	*TA 1535*	*TA 100*	*TA 1537*	*TA 98*	*WP2uvrA*
Without S9 mix					
iprovalicarb 0 (DMSO)	*22*	*92*	*18*	*29*	*48*
10	*25*	*92*	*19*	*27*	*47*
33	*21*	*94*	*17*	*34*	*46*
100	*24*	*91*	*17*	*28*	*43*
333	*25*	*82*	*16*	*29*	*39*
1000	*25 p*	*91 p*	*15 p*	*27 p*	*49 p*
2500	*25 p,m*	*87 p,m*	*17 p,m*	*26 p,m*	*36 p,m*
5000	*26 p,m*	*88 p,m*	*15 p,m*	*31 p,m*	*44 p,m*
Na-azide 10	*1723*	*1979*	*-*	*-*	*-*
4-NOPD 10	*-*	*-*	*-*	*379*	*-*
4-NOPD 50	*-*	*-*	*86*	*-*	*-*
MMS 3 µl	*-*	*-*	*-*	*-*	*-*
					515
With S9 mix					
iprovalicarb 0 (DMSO)	*31*	*126*	*27*	*38*	*55*
10	*38*	*111*	*36*	*44*	*50*
33	*35*	*113*	*26*	*42*	*71*
100	*34*	*123*	*23*	*45*	*65*
333	*33*	*122*	*25*	*48*	*59*
1000	*31*	*128 p*	*26 p*	*46 p*	*50 p*
2500	*28 p,m*	*102 p,m*	*19 p,m*	*36 p,m*	*48 p,m*
5000	*29 p,m*	*98 p,m*	*13 p,m*	*26 p,m*	*46 p,m*
2-AA 2.5	*300*	*1737*	*227*	*1482*	*-*
2-AA 10	*-*	*-*	*-*	*-*	*253*

P: precipitate; m: manual count

III CONCLUSION

In the absence of any increase in mutant counts, there was no indication of any mutagenic effect of iprovalicarb in this test. Iprovalicarb was therefore considered to be non-mutagenic in the Salmonella typhimurium and Escherichia coli reverse mutation assay.

Document M / Tier 2, KIIA 5 Toxicological and Toxicokinetic Studies on the Active Substanceon Iprovalicarb
(Submission for Annex I Renewal)

Report:	[redacted];2001;M-071975-01
Title:	SZX 0722 - In vitro chromosome aberration test with Chinese hamster V79 cells using 18 hours treatment without S9 mix
Report No:	31333
Document No:	M-071975-01-1
Guidelines:	***Deviation not specified***
GLP/GEP:	***Yes***

Executive summary:

In this in vitro assessment of the clastogenic potential of iprovalicarb (Batch-No:898222005; 96.8% purity), Chinese Hamster V79 cells were exposed to SZX0722 at 30, 60, 120 and 240 µg/mL (18 hours treatment) diluted in dimethyl sulfoxide (DMSO) in the absence of an Aroclor 1254-induced rat liver metabolic activation system (S9 mix). Cultures of all concentrations were harvested 18 hours after treatment start. Concentrations were selected for metaphases reading on the basis of their cytotoxicity and precipitation in the medium. Adequate positive control (mitomycin C) was also used.

Without S9 mix, cytotoxic effects were observed at 120 µg/mL and above. Precipitation in the medium occurred at 120 µg/ml and above. Therefore, concentrations of 60, 120 and 180 mg/ml were chosen for reading.

None of the cultures treated with SZX0722 in the absence of S9 mix showed biologically relevant or statistically significant increased numbers of aberrant metaphases.

The positive control mitomycin C induced clastogenic effects and demonstrated the sensitivity of the test system. Iprovalicarb was considered not to be clastogenic for mammalian cells in vitro.

1. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:
Description: ***white lumpy powder***
Lot/Batch: ***898222005***
Purity: ***96.8%***
CAS: ***140923-17-7***
Stability of test compound: ***Stable for the duration of the study***

2. Control materials: Negative: ***Culture medium***
Solvent: ***DMSO for SZX0722 and Hanks's balanced salt solution for positive controls (Seromed)***
Positive: ***Mitomycin C (Fluka, batch 410943/1 31600) without S9 mix at 0.03 µg/mL for a treatment period of 18 hours.***

3. Test organisms:
Cell line: ***Chinese hamster V79 lung cells***
Source: ***Cells obtained from [redacted], Darmstadt in 1993, stored in the laboratory since then.***

4. Test compound concentrations:
SZX0722 was used without S9 mix up to 240 µg/mL.

B. STUDY DESIGN AND METHODS:

The experimental phase of the study was performed from August 1 to August 17, 2001 at Bayer Healthcare AG (PH-PD P Health Care Toxicology).
The in vitro cytogenetic test is a mutagenicity test system for the detection of chromosome aberrations in cultured mammalian cells. The test is designed to detect structural aberrations (chromatid and chromosome aberrations) in cells at their first post-treatment mitosis.

1. Determination of cytotoxicity

Cytotoxic effects of the test substance were assessed in the pre-test as well as in the main-study. Cell survival as well as mitotic index were determined. At the end of the respective incubation period cells of all cultures of the respective period were trypsinized, and an appropriate dilution was counted using a hemocytometer (improved Neubauer) to determine cell survival.
The mitotic index was determined for all cultures. The number of mitotic cells among a total of 1000 cells per culture was determined. All cells which were not in interphase were defined as mitotic.
In the main study, cultures with a total incubation period of 8 hours were additionally and exclusively used to determine the cytotoxicity of SZX 0722.

2. Treatment protocol

The general protocol of the test system was similar to published procedures (e.g. Dean and Danford, 1984). Chinese hamster V79 cells were passaged on the day prior to treatment. Approximately 1 x 106 cells were seeded in 20 ml of medium per 75 cm2 flasks and incubated as described in section 4.2.2. All cultures were set up in duplicate.
Immediately before treatment, the medium was removed from the cultures.
For the treatment, the following fresh solutions were added to each flask: components volume in ml medium containing 2% PCS test substance solution. The cells were incubated for 18 at 37°C as described above. 0.2 ml Colcemid solution (40 ug/ml water) were added to each flask two hours prior to the end of the incubation period to arrest the cells in a metaphase-like stage of mitosis (metaphase).
Positive control and solvent control (0.2 ml solvent per culture), were set up in parallel and handled as described for SZX 0722-treated cultures. Solvent controls are used as negative controls.

3. Chromosome preparations:

After the removal of the medium from each flask, the cells were trypsinized, suspended in medium and centrifuged for approximately 5 minutes at 700 rpm. The supernatant was removed and 1 to 2 mL of a hypotonic solution (0.4% KCl; 37°C) was added to each tube. Within 4 minutes, the volume was brought to 6 mL with additional hypotonic solution and cells were resuspended. The cells were centrifuged again and fixed with cold (4°C) fixative (ethanol/acetic acid 3:1) for 20 minutes at room temperature. Cells were pelleted and resuspended in fixative as before and centrifuged again. The pelleted cells were resuspended in a small volume of fixative and the suspension was dropped onto clean slides. The slides were allowed to dry for at least 2 hours. Thereafter, they were submerged in pure methanol for 3 minutes and stained for 15-20 minutes in 3% Giemsa solution. Slides were rinsed twice in water and once in acetone and were then kept in xylene for about 30 minutes. The slides were allowed to dry completely and covered. At least two slides were generated per culture.

4. Evaluation criteria:

Coded slides were evaluated using a light microscope at a magnification of about 1000. Chromosomes of approximately 200 metaphases per concentration, 100 metaphases from each of two parallel cultures, were examined. Only metaphases containing the modal chromosome number (22) were analyzed unless exchanges were detected. The following aberrations were recorded: gaps (an achromatic lesion within a chromatid arm without dislocation of the chromatid end), break (a discontinuity of one chromatid with dislocation of the chromatid end), fragment (part of chromosome without centromer), deletion (result of a break with the terminal chromatid part of the chromosome missing within the metaphase under assessment), exchange (exchange of chromatid-parts between different chromosomes or within the same chromosome), multiple aberration (when five or more structural changes occur within one metaphase). Observed polyploidy metaphases were recorded but not used for assessment.

5. Assessment criteria:

An assay was acceptable, if there was a biologically relevant increase in chromosome aberrations induced by positive controls and if the numbers of aberrations for the negative controls were in the expected range.
An increased incidence of gaps of both types without a concomitant increase of other aberration types was considered not to be an indication of a clastogenic effect.
A test was considered positive, if there was a relevant and statistically significant increase in the aberration rate.
A test was considered negative, if there was no such increase at any time interval or if there were statistically significant values, which were, however, within the range of historical negative controls.
A test was considered equivocal, if there was an increase above the range of historical negative controls which was statistically significant but considered not relevant, or if an increase occurred, which was considered relevant, but which was not statistically significant.

II. RESULTS AND DISCUSSION

A. Mitotic and survival indices:

Mitotic index: in absence of S9 mix, the mitotic index was reduced at 120 µg/mL and above (tables 5.4.2-1 and 5.4.2-2). The cultures treated with mitomycin C showed no reduction in mitosis rate compared to solvent control.

Survival index: Survival indices were significantly reduced at 240 µg/ml.

B. Chromosome aberrations:

The results of the microscopic evaluation of the metaphases for structural chromosome aberrations are presented in Table 5.4.1.7-1. Numbers of aberrations (listed by class) and numbers of metaphases with aberrations including and excluding gaps and numbers of metaphases with exchanges for individual cultures are listed (in percent).

No biologically relevant and statistically significant increases of numbers of metaphases with aberrations were detected (Table 5.4.1.7-1).

The treatment with the positive control mitomycin C resulted in a clear and statistically significant increase of metaphases with aberrations and demonstrated the sensitivity of the test system.

Table 5.4.1.7-1: Summary of the results of the chromosome aberration study with SZX0722 (18 hours treatment)

Experiment	*Harvest time*	*Test item concentration µg/ml*	*Mitotic indices (% of control)*	*Aberrant cells Incl; gaps*	*Aberrant cells Excl. gaps*	*Aberrant cells With exchange*
	18 h	*DMSO*	*100.0*	*1.0*	*1.5*	*0.5*
		Mitomycin C	*109.9*	*26.5***	*24.5***	*7.0***
		60	*96.5*	*1.0*	*1.0*	*0.0*
		120	*93.0*	*0.0*	*0.0*	*0.0*
		180	*83.2*	*2.0*	*2.0*	*0.5*

** p < 0.05, ** p < 0.01*

III. CONCLUSION

After 18 hours treatment of Chinese hamster V79 cells with SZX 0722 concentrations of 60, 120 and 180 ug/ml were used for assessment of the clastogenic potential of SZX 0722.
None of these cultures treated with SZX 0722 showed statistically significant or biologically relevant increases of numbers of metaphases with aberrations.
The positive control mitomycin C induced clear clastogenic effects and demonstrated the sensitivity of the test system.
Based on the results of this test, SZX 0722 is considered not to be clastogenic for mammalian cells in vitro.

KIIA 5.4.2 - In vivo genotoxicity

Report:	[redacted];1995;M-000300-01
Title:	SZX 0722 - Micronucleus test on the mouse
Report No:	24016
Document No:	M-000300-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];1998;M-001273-01
Title:	SZX 0722 - 32 P-Postlabelling assay in female rat uterus and urinary bladder epithelium in vivo
Report No:	27184
Document No:	M-001273-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.5 - Long-term toxicity and carcinogenicity

Summary of long-term toxicity studies

Table 5.5-1: Summary of long-term toxicity studies

Type of study	Species	Dose range tested	NOAEL (mg/kg/day)	Reference
chronic/carcino-genicity, oral	rat	0-500-5000-20000 ppm	500 ppm (26.0)	[redacted], 1998, 27160
oncogenicity, oral	mouse	0-280-1400-7000 ppm	280 ppm (58.5mg/kg /day)	[redacted], 1997, 26450

The 2-year rat chronic/carcinogenicity study showed higher liver weight (+20 % in females) associated with hepatocellular hypertrophy (20/50) in females at 20,000 ppm, slight increased incidence of rare benign tumours (uterus, urinary bladder, clitoral gland, bones) at 20,000 ppm . The NOAEL was established at 500 ppm (26 mg/kg/d). Given that the increased incidence of tumors at 20,000 ppm was minimal and close to the historical control data, these tumors have been considered not to be treatment-related. In the mouse oncogenicity study, the liver and the kidneys were the target organ. The NOAEL was established at 280 ppm (58.5 mg/kg/d). In both species, iprovalicarb was not carcinogenic.

The increased incidences of tumors have been investigated in specific mechanism studies mentioned in section 5.5.4 below.

Please refer to point IIA 5.5 (EU point 5.5) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.5.1 - Long-term (2 years) oral toxicity in the rat

Report:	[redacted];1998;M-001236-01
Title:	SZX 0722 - Chronic toxicity and cancerogenicity investigations in Wistar rats (administration in the feed over 24 months)
Report No:	27160
Document No:	M-001236-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.5.2 - Carcinogenicity study in the rat

Report:	[redacted];1998;M-001236-01
Title:	SZX 0722 - Chronic toxicity and cancerogenicity investigations in Wistar rats (administration in the feed over 24 months)
Report No:	27160
Document No:	M-001236-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.5.3 - Carcinogenicity study in the mouse

Report:	[redacted];1997;M-000405-01
Title:	SZX 0722 - Oncogenicity study in B6C3F1-mice (Administration in the food over 2 years)
Report No:	26450
Document No:	M-000405-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.5.4 - Mechanism of action and supporting data

A specific mechanism study has been conducted in Wistar rats to determine the tumor initiation potential of iprovalicarb administered by oral gavage at 1,000 mg/kg for 28 days. The study showed no difference between control and treated group in the number of foci of altered hepatocytes suggesting that iprovalicarb was devoid of any initiation potential and does not possess a carcinogenic potential.

The following additional information in the area of mammalian toxicology will be included in the dossier supporting the approval renewal: A new mechanistic study (M-267627-01-1 and M-422060-01-1) has been performed to assess cell proliferation in tissues of concern for carcinogenesis in the Wistar rat carcinogenicity study: bones, thyroid, uterus & urinary bladder. On the slides from the rat carcinogenicity 104-week sacrifice time, the immunohistochemical determination of Proliferating Cell Nuclear Antigen (PCNA), an endogenous marker for cell replication (G1, S phases of cell cycle), showed the absence of biologically significant differences between DNA replication in control and treated tissues for osteocytes, thyroid follicular cells, uterine cells & urothelial cells: the slight increased incidence of tumors are not related to treatment with iprovalicarb. Iprovalicarb is thus devoid of any carcinogenic potential.
An additional position paper (M-461421-02-1) has been provided as well by the external expert consultant ([redacted]) to address a question raised by the RMS during the review of the

PCNA evaluation of the 2-year rat carcinogenicity tissues on the biological relevance of the increased replication fraction in the uterus and urinary bladder.

During the sub-chronic feeding study in rats at 500 ppm and 20,000 ppm, plasma samples were investigated to determine the toxicokinetics of iprovalicarb and the main metabolite SZX0722-carboxylic acid (*M03)*.

An additional sub-acute toxicity study was conducted in rats with thermodynamically stable and thermodynamically labile modification of iprovalicarb in diet for 2 weeks at 2,000 and 20,000 ppm. The modifications are a result of molecular polymorphism, leading to two crystal modifications of different stability. However, no toxicologically relevant differences in plasma levels of the main metabolite SZX0722-carboxylic acid (*M03)* were observed.

Please refer to point IIA 5.8 (EU point 5.8) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL from July, 2002)".

Report:	[redacted];1997;M-001057-01
Title:	[14C] SZX 0722: Investigation of the biokinetic behaviour and the metabolism in the rat following subchronic feeding
Report No:	PF4322
Document No:	M-001057-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1998;M-001271-01
Title:	SZX 0722 - Investigation of plasma concentrations in rats in a subchronic feeding study (study no. T 9 061 633) Annex to M 718190/62
Report No:	27238
Document No:	M-001271-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];[redacted];1998;M-001275-01
Title:	SZX 0722 - Bioavailability study comparing of thermodynamically stable against thermodynamically labile modification (feeding study over 2 weeks in rats)
Report No:	27237
Document No:	M-001275-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];1998;M-001273-01
Title:	SZX 0722 - 32 P-Postlabelling assay in female rat uterus and urinary bladder epithelium in vivo
Report No:	27184
Document No:	M-001273-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1993;M-000347-01
Title:	SZX 0722 - Liver foci test for initiating effect
Report No:	22120
Document No:	M-000347-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**no**

Report:	[redacted];[redacted];[redacted];*2006;M-267627-01*
Title:	***Proliferating cell nuclear antigen (PCNA) immunohistochemical evaluation report on selected target tissues from SZX072: Chronic toxicity and carcinogenicity investigations in Wistar rats (administration in the feed over 24 months) ...***
Report No:	***T1055425***
Document No:	***M-267627-01-1***
Guidelines:	***Not specified***
GLP/GEP:	***no***

Report:	[redacted]*2012;M-422060-01*
Title:	***Expert panel consensus report in assessing the weight of evidence of iprovalicarb carcinogenicity in rats and mice.***
Report No:	***010512***
Document No:	***M-422060-01-1***
Guidelines:	***Not specified***
GLP/GEP:	***no***

Executive summary

Iprovalicarb was administered in the feed to groups of 50 Wistar (Hsd/PIN:WU) rats/sex/dose level for 24 months at dose levels of 0, 500, 5000, or 20000 ppm (equivalent to 0, 26, 263, or 1110 mg/kg/day in males and 0, 32, 326, or 1380 mg/kg/day in females). This study, entitled "SZX0722: Chronic toxicity and carcinogenicity investigations in Wistar rats (administration in the feed over 24 months)" was reported on February 4, 1998 ([redacted]1998, M -001236-01-1, see section 5.5.2).

In order to monitor the rate of proliferation in certain target tissues, proliferating cell nuclear antigen (PCNA) immunohistochemical stain was performed on selected tissues from archival material (blocks) from the 12-month and 24-month sacrifices. The PCNA results from the 24-month tissues have been reported and the weight-of-evidence of all of the significant rat neoplastic findings has been assessed (see document M-267627-01-1). This expert panel consensus document (M-422060-01-1), reports the analysis of PCNA staining of the 12-month tissues following a requirement made by US-EPA and confirms that the neoplasms observed in rats following a 2-year treatment-period with iprovalicarb are not relevant for humans. In conclusion, iprovalicarb is clearly considered as a non-carcinogenic compound.

Introduction

Proliferating cell nuclear antigen (PCNA) immunohistochemical reaction was performed on bone and cartilage tissues from males and urinary bladder (UB), uterus and thyroid tissues from females from archival material (blocks) from the 12- (interim) and 24-month final terminations from randomly selected samples per group of rats, including the samples with neoplasms. In late 2007, an IPRO Expert Panel was convened consisting of [redacted] replaced by [redacted] (Rapporteur) and [redacted]. Using a Weight of Evidence (WoE), the Panel concludes that the tumors observed in the 4 tissues of male and female rats were not treatment related.

Results and Discussion

In high dose (HD) male rats only (three osteosarcomas), there was a statistically significant (p<0.01) positive trend for osteosarcoma in two combined bone sites, and also significant (p<0.05) by pair-wise compari[redacted] (HD vs control). The incidence exceeded the concurrent control incidence, and historical control data at both Bayer and RITA databases. These tumors are rare (< 1%) and malignant.

a. iprovalicarb is non-genotoxic and accordingly, for an epigenetic agent proliferative, hyperplastic or preneoplastic changes would be expected to precede tumor development. However, no evidence of increased osteoblastic activity resulting in hyperostosis or other preneoplastic findings was present.

b. At the HD level females received ~20% higher systemic exposure to iprovalicarb and are more prone to compensatory hyperostosis in old age (because of estrogen reduction) than male rats, yet no osteosarcomas were evident in females.

c. The occurrence of the three osteosarcomas was not time-related, i.e., one at 53, one at 89, and one at 94, but none at 106 weeks. This pattern of occurrence (41 weeks apart and not at the end) is the pattern of a background ("spontaneous") neoplasm.

Thus, the Expert Panel WoE assessment based on the points made above, concludes that the 3 osteosarcomas observed in HD male rats were not related to iprovalicarb treatment.

In mid dose MD (2/48 or 4.2%) and HD (3/50 or 6%) female rats, there were increases in the incidences of combined follicular cell adenomas and carcinomas of the thyroid. There was a statistically significant trend (p<0.05), but pair-wise compari[redacted] with controls was not significant. These tumors exceeded the concurrent control and historical control data in the Bayer and RITA databases. Subsequent re-evaluation of all follicular cell neoplasms, found that one mid dose adenoma was very small, was reclassified as focal hyperplasia, and was disqualified as a neoplasm.

d. Neither the trend analysis nor the pair-wise compari[redacted] achieved the statistical significance required to establish a treatment related effect, i.e., p<0.01 for pair-wise compari[redacted] and p<0.005 for trend analysis (US FDA, 2001).

e. Iprovalicarb is non-genotoxic and accordingly proliferative, hyperplastic or preneoplastic changes would be expected to precede tumor development. Increased follicular cell proliferation is always present with treatment with known thyroid non-genotoxic carcinogens (Capen et al, 1999; Rice et al, 1999). The thyroid histopathology, including lack of evidence of increased trophicity or activation, and lack of increased follicular cell rate of proliferation, measured by PCNA at 12 and 24 months, and lack of a significant incidence of preneoplasia (hyperplasia) indicate that iprovalicarb lacks the capability of inducing any of these effects, even in the HD where three follicular cell neoplasms were found. There was no reduction in latency for tumor development, the only neoplasm identified prior to termination was a HD adenoma observed at 82 weeks in a moribund rat.

Thus, the Expert Panel WoE assessment, based on the points described above, concludes that follicular cell neoplasms of the thyroid are not related to iprovalicarb. Furthermore, the mode of action for non-genotoxic carcinogens involves hypothyroidism produced by a variety of mechanisms, and this MoA has been considered not to be relevant to humans (Rice et al, 1999; Capen et al 1999).

In mid dose and high dose female rats the numerically greater incidence of mixed Müllerian uterine malignant tumors, 1/48 or 2.1% and 2/50 or 4%, respectively, was not statistically significant by either trend analysis or by pair-wise compari[redacted] to controls. The tumor incidence, however, did exceed the historical control incidence in both Bayer and RITA databases.

f. There was a lack of statistical significance for both trend and pairwise comparisons.

g. iprovalicarb is non-genotoxic and accordingly proliferative, hyperplastic or preneoplastic changes would be expected to precede tumor development. Currently, assessment of uterine histopathology and uterine rate of cellular proliferation, including luminal/stromal/ myometrial cellular elements measured by PCNA at the 12-month interval, did not yield evidence of increased trophicity, dysplasia or preneoplasia.

h. Organ weights of hormonally sensitive organs provided no evidence of pro- or antiestrogenic effects indicative of any disruption of uterine hormonal homeostasis.

Thus, the Expert Panel WoE assessment based on the points made above concludes that the three Müllerian tumors observed in one mid dose and two high dose rats are not related to iprovalicarb treatment.

In high dose females (2/50 or 4%), a numerically greater incidence of benign transitional (urothelial) cell papillomas of the urinary bladder was present, but the increase was neither statistically significant by trend analysis, nor by pair-wise compari[redacted]*with controls.*
Their incidence exceeded the concurrent and historical controls, and the Bayer and RITA databases.

*i. These two **benign** neoplasms were not statistically significant.*

j. The neoplasms were observed in HD female rats only at termination (106 weeks) and no malignant tumors were present. Such findings do not strictly qualify to label a compound as "carcinogenic", since no malignant urinary bladder urothelial cell tumors were present.

k. iprovalicarb is non-genotoxic and accordingly proliferative, hyperplastic or preneoplastic changes would be expected to precede tumor development, especially when both urothelial cell tumors were benign (at 106 weeks). Currently, assessment of urothelial cell histopathology, indicates lack of evidence of chronic irritation, hyperplasia, dysplasia, preneoplasia and lack of increased urothelial cell proliferation at the 12-month interval, indicating that the two benign tumors observed at 106 weeks in high dose females, are not causally related to iprovalicarb exposure. Furthermore, there was not an increased incidence of hyperplasia at the termination which would be expected if these papillomas were treatment related.

Thus, the Expert Panel WoE assessment based on the above points, concludes that the UB benign tumors are not related to iprovalicarb treatment.

Conclusions

A Weight of Evidence analysis was applied to the four identified neoplasm types in four tissues, osseous (malignant only in 3 HD male rats), uterine (malignant only in three HD rats), follicular cell tumor of the thyroid (benign in two HD females, and malignant in one MD and one in HD females), and urothelial cell papilloma of the urinary bladder (two benign in HD females).

Iprovalicarb was concluded to be non-genotoxic and this was supported by structure activity analysis. Marginal statistical significance for a treatment related effect was achieved only for the osseous tumors. For this and the other three tumor types there was no evidence for tissue changes or preneoplasia in advance of tumors and no increased incidences of preneoplastic lesions at the terminal euthanasia which are expected for a non-genotoxic carcinogen. Based on such considerations, the Expert Panel concludes that these tumors were not treatment related.

Using the WoE, the Expert Panel concludes that there is no evidence of iprovalicarb-induced carcinogenicity in male and female Wistar rats.

Report: [redacted];[redacted];*2013;M-461421-01*
Title: *Iprovalicarb PCNA reporting*
Report No: *M-461421-01-1*
Document No: *M-461421-01-1*
Guidelines: ***not specified***
GLP/GEP: ***n.a.***

Executive summary:

The rapporteur questions the criteria for a biologically relevant versus a non-biologically relevant response in the PCNA assay with regard to findings in the uterus and urinary bladder. For both of these two tissues, the interpretation of a lack of biological significance was based on the fact that the RF value increases, were marginal and below the approximate 3-fold increase over the value of the appropriate controls, which we have found to be a threshold for clear biological significance.

Introduction

The uterine tissue is a composite of stromal (~ 51%), which also includes the glandular compartment, the luminal epithelial (~ 48%), and myometrial (~ 1%) cellular compartments. Thus, the uterine tissue has cells with different patterns of proliferation after maturity, i.e. expanding, e.g., myocytes and stromal cells; indeterminate e.g., endocrinocytes; and renewing cell types, e.g., mucosal epithelia and ductular cells ([redacted], 1996). In addition, in most tissues the maintenance of adequate trophicity is assumed by the parasympathetic nervous system. The exceptions are the hematopoietic, pancreatic, and uterine tissues which have the sympathetic nervous system assume maintenance of trophicity, and which is aided by estrogens. Any interruption of trophicity, triggers stimulation of proliferation ([redacted], 1994). Endometrial hyperplasia is found as a spontaneous change in rats as age advances. Hyperplasia can either be diffuse or focal (polypoid). In aging rats, cystic and proliferative changes are also found in association with polyps ([redacted], 2012).

Results and Discussion

In the present rat bioassay with iprovalicarb at twelve months, similar slight numerically greater values for replicating fractions (RF) were observed in all doses (LD, low, MD, mid, and HD high doses) of iprovalicarb i.e., 1.1- to 1.2-fold (Table 1). Only the mean HD value was significant (at $p < 0.05$) in pair-wise comparisons to controls, although the RF increase was only ~ 1.2-fold greater than control. However, the absence of a dose response calls into the question whether the values reflect a compound-induced effect. Moreover, routine microscopic evaluation of the same uterine tissues did not reveal either diffuse or focal areas of hyperplasia, or even microscopic evidence of disruption of trophicity (hypertrophy or atrophy, even dystrophy) in any of the cellular compartments of the uterus. At the 24 month termination, slightly higher significant increases, i.e., 1.5- to 1.9-fold were observed. The relevance of these is questionable, however, since they were found only at the end of the study and hence cannot be implicated in the development of the uterine neoplasms.

Table 1. Effect of exposure to iprovalicarb (IPRO) on the replicating fraction (RF) of uterine and urothelial non-neoplastic (NON) and neoplastic (NE) tissues in Wistar rats compared to concurrent controls at 12- and 24-month terminations [a].

	12 Month interim termination			
	Uterine [b]		Urothelial	
Group identification	RF fold change [c]	Neoplasms at 24 months	RF fold change	Neoplasms at 24 months
			NON	
Control	CV	NA	CV	NA
LD IPRO [d]	~ 1.1	NA	~ 1.1	NA
MD IPRO [d]	~ 1.0	NA	---	NA
HD IPRO [d]	~ 1.2 *	NA	~ 1.2	NA
	24 Month interim termination			
	Uterine		Urothelial	
Control	CV	NA	CV	NA
LD IPRO [d]	~ 1.5 *	NA	TNE	NA
MD IPRO [d]	~ 1.6	1 MMMT [e]	TNE	NA
HD IPRO [d]	~ 1.9 *	2 MMMT [f]	~ 2.0 *	2 UP [g]

[a], Adapted and modified from [redacted] et al, 2012; [b], a composite of stromal (~51%), luminal (~48%) and myometrial (~1%) cells; [c], fold increase over the controls; [d], low (LD), mid (MD), or high dose (HD) of iprovalicarb in the diet; [e], one malignant mixed Müllerian (MMMT) tumor, observed at final termination; [f], two MMMTs observed at final termination, all three MMMTs had different pattern of development (e.g., metastasis) and lacked significance for both trend and pair-wise comparisons; [g], two benign focal urothelial papillomas (UP) observed at final termination, showing lack of statistical significance,*, significant at $p<0.05$ in a pairwise compari[redacted] to controls; CV, control value of the RF of the controls which served to calculate the RF fold change; NA, not applicable; TNE, tissue was not evaluated; ---, same as in controls.

In other studies in our laboratory with the selective estrogen-receptor modulators tamoxifen (TAM) and toremifene (TOR), and the pro-estrogen, diethylstilbestrol (DES), we studied the biological and statistical significance of a variety of changes (increases or decreases) in each of the cellular

compartments of the uterine tissue (Table 2). As a result, the biological fold changes above or below ~ 3-fold seem to be more useful than the mean (± SD) RF values of exposure compared statistically to either pair-wise or trend comparisons. This was confirmed by the lack of hyperplasias with TAM or TOR, and the presence of hyperplasia with DES, which also induced metaplasia in the same compartments ([redacted] et al, 1998).

Table 2. Effect of exposure to tamoxifen, toremifene, or diethylstilbestrol on the replicating fraction (RF) of different uterine cellular compartments in F344 rats compared to concurrent controls [a]

RF fold [b] change in uterine cellular compartments	Group identification			
	Control	TAM [c]	TOR [c]	DES [c]
Luminal epithelium	CV [d]	~ 3.0 ↓	~3.4 ↓	~2.2 ↑
Glandular epithelium	CV [d]	~1.4 ↑	~1.3 ↑	~ 2.0 ↑
Stroma	CV [d]	~ 0.6 ↓	~ 0.5 ↓	~7.0 ↑
Myometrium	CV [d]	~0.4 ↓	~0.3 ↓	~ 8.0 ↑

[a], Adapted and modified from [redacted] et al, 1998; [b], fold increase (↑) or decrease (↓) over controls; [c], given daily by gavage for 12 weeks; [d], control value of the RF of the controls, which served to calculate the RF fold change.

The three (one in MD and two in HD iprovalicarb) uterine mixed Müllerian cell malignant neoplasms (MMMT) were observed at the final termination (MD, 107 weeks; HD, 105 and 106 weeks) (Table 1). Each of these neoplasms had a different histogenesis. Namely, the MD neoplasm was accompanied by a stromal uterine focal polyp, and a luteoma in the ovary, without any evidence of metastasis. One HD neoplasm (at 106 weeks) had no other neoplasms in any of the tissues. The other HD neoplasm (at 105 weeks) had evidence of extensive metastasis to the ovary, kidney and lung. Yet, at termination, the RF fold increase was ~ 1.6-fold in MD and ~ 1.9-fold in HD. Both fold increases were similar (~ 1.5-fold) to the LD increase which had no MMMT neoplasms (Table 1). Hence, these three neoplasms were not causally linked to any disruption of trophicity based on epigenetic effects induced by iprovalicarb.

The other tissue of concern, urothelial tissue, is a renewing cell type tissue having the capacity for the highest pattern of proliferating response ([redacted] and [redacted], 1996). In the rat, there is absence of tight junctions rendering the superficial urothelial layer ineffective as an intraluminal barrier ([redacted], 1975) and making the rat urinary bladder very sensitive to intraluminal changes ([redacted] et al, 1994). Intraluminal changes in pH (both extremes) produce simple hyperplasia, which, with persisting stimuli becomes adaptive, reparative, and finally preneoplastic hyperplasia, which is irreversible. Cellular atypia is also present in this preneoplasia hyperplasia, which can occur in small areas of papillary, nodular or mixed hyperplasias ([redacted] et al, 1994). In the present rat bioassay, at 12 months, the fold changes (increase) in RF were minimal, i.e. 1.1- and 1.2-fold, without statistical significance (Table 1). Routine microscopy did not reveal any hyperplasia of any kind. The picture remained the same at 24 months, although the HD RF value was significantly elevated ($p < 0.05$), but the RF fold increase was only ~2 (Table 1). Again, between 12 and 24 months, no hyperplasia of any kind was present, and only two HD rats had benign urothelial cell papillomas. These two rats had no evidence of calculi or urothelial irritation. Thus, the presence of only two benign tumors (not statistically significant), without compound-related evidence of chronic urothelial irritation and subsequent dose-related preneoplastic hyperplasia cannot be causally linked to iprovalicarb epigenetic effects.

We can find no published criteria for biologically significant increases. Our data as described above shows that 3-fold increase is biologically significant. Dr. S. Cohen of the University of Nebraska, an expert in this matter (Cohen and Ellwein, 1990), concurs that there are no published criteria and states that a 2-fold has frequently been used. All of the relevant 12 month RF levels were below a 2-fold increase. We know of no evidence that less than a 2-fold increase can drive the carcinogenic process. In fact, [redacted] et al (1994) reported on three noncarcinogenic organophosphate pesticides which produced up to 1.4-fold increases over controls in rat liver which were considered not to be increases. [redacted] et al (1993) found statistically significant increases in cell proliferation in nontarget organs of carcinogenicity which were considered "nonspecific" and "without biological significant". Also in the urinary bladder, carcinogens were associated with elevation of labelling index of 1.78 to 9.31.

In any event, no tumors were found in low dose rats (32 mg/kg body weight) and this dose may be considered to be below a threshold for a nongenotoxic agent.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:

Description:	***SZX 0722***
Lot/Batch number:	***05013/0194***
Purity:	***98.8% - 98.5% a.i. (w/w)***
Test material Stability:	***Stable at 25 ± 5°C / room temperature***
Molecular weight:	***320.4 g/mol***

2. Vehicle and /or positive control: None

3. Test animals:

Species:	***Rat***	
Strain:	***Wistar Hsd/WIN: WU***	
Age / weight at study initiation (Day 1):	***4-5 weeks old / 129 g mean group weight for males; 105 g mean group weight for females***	
Source:	[redacted]***, Germany.***	
Housing:	***by sex in groups of 5 unless reduced by mortality or isolation. The cages were suspended, stainless steel and wire mesh.***	
Diet:	***Altromin 1231, ad libitum except at designated time periods***	
Water:	***Filtered and softened tap water from the municipal water supply, ad libitum***	
Environmental	***Temperature:***	***20-24 °C***
	Humidity:	

conditions:	*Air changes:*	*55 ± 5 %*
	Photoperiod:	*15 to 20/hr*
		12 hrs dark/ 12 hrs light (6 am- 6 pm)
Acclimatization period:	*15 days*	

B. STUDY DESIGN:

1. In life dates: *January 24, 1994 to February 09, 1996, at [redacted] Germany.*

2. Animal assignment and treatment

Male and female experimental animals were randomized prior to the start of application and allocated to experimental groups. For this purpose, the animals were weighed individually, classified according to their weight (light and heavy), and, separated by sex, placed into huge tubs where they could spread at random. Using a random list based on evenly distributed chance numbers, which was especially generated for this study, animals were taken and allocated to the group and animal number specified by the random list, with the animals being placed one after the other into cages with ascending numbers.

3. PCNA evaluation

Staining for proliferating cell nuclear antigen (PCNA) was performed at [redacted], Germany.

The tissue samples were obtained exclusively from archived material (blocks) of the 2-year carcinogenicity study ([redacted], 1998) via a study protocol amendment. For the organs/tissues of interest, randomly selected tissue samples and neoplasms were processed.

Paraffin sections of the urinary bladder, uterus, thyroid gland, bone, cartilage and tumor samples about five microns thick were cut and de-paraffinized. For gentle heat pretreatment, 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 pretreated 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 visualized by addition of 3,3 diamino-benzidine tetrachloride (DAB) and the enzyme substrate hydrogen peroxide. Finally, the sections were counterstained with Hematoxylin. 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 400X 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 (UB, uterus, thyroid) were counted. Again, the most proliferative areas were selected for counting. Based on the above, the labeling indices of each tissue were calculated, reflecting the percent replicating fraction (RF).

All tissues selected rendered successful PCNA staining even when the tissue stained negative, as was the case with bone and cartilage cells which are stable and do not replicate. The white blood cells in vessels surrounding bone tissue were PCNA positive as was the bone marrow.

For statistical comparisons of each exposed group to controls, the Student-t-test was used.

II. *RESULTS*

In male 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, RF range 13 to 23 (Table 5.5.4.6-1). The percent RF within 2 osteosarcomas was 28 in No. 203 (tumor in the lower jaw, which was lethal to the rat on week 53) and 38 in No. 220 (tumor 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 5.5.4.6-2).

In females, the percent RF group means of the uterine tissue showed an exposure- related increase (Table 5.5.4.6-1). 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) (Appendix 1). Likewise in females, the percent RF group mean of group 4 (20000 ppm) of UB urothelial tissue showed a significant increase compared to controls (Table 5.5.4.6-1). The percent RF within the 2 urothelial (adenomas) papillomas was 80 in No. 431, and 50 in No. 455, both killed at terminal sacrifice. The percent RF group mean of the nonneoplastic follicular thyroid tissue from all 3 exposure groups was similar to control (Table 5.5.4.6-1). 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 5.5.4.6-2, Appendix 1 from study report).

The occurrence of neoplasms in all rats dying early (i.e. during the first 18 months of the study or 2/3rds of the study duration) is given in Table 5.5.4.6-3. This study was terminated at 106-107 weeks and had a 12 month interim sacrifice. Four rats were sacrificed moribund (Nos. 432, 148, 203 and 426) before or during the interim sacrifice. All four of the early deaths had lethal neoplasms; No. 432, pituitary adenoma; No. 148, malignant brain astrocytoma; No. 203, osteosarcoma of the lower jaw, and No. 426 adenocarcinoma of the uterus (Table 5.5.4.6-3). All common neoplasms and the majority of the early neoplasms are consistent with the pattern of early background (spontaneous) neoplasia. Tumors in 3 of the 4 affected tissues (bone/cartilage, uterus, and thyroid gland) occurred before the 60th week of the study. Only the neoplasms of the urothelium of the urinary bladder occurred much later. The bone/cartilage was identified as the sole target site of neoplasia in males, and the uterus, thyroid and urinary bladder were identified in females.

Table 5.5.4.6-1. Mean Percent Replicating Fraction (RF) Values[a] of Selected Tissues from Rats Exposed to IPRO[b]

Group Identification and Sex	*Uterus*		*Thyroid*		*Urinary Bladder*		*Bone / Cartilage*	
	Neo[c]		*Neo*		*Neo*		*Neo*	
Group 1- Control								
Males	*NSA*	—	—	—	—	—	*CBE*	*NA*
Females	*28.53 ± 5.32*	*NA*	*1.76 ± 0.64*	*NA*	*15.59 ± 4.36*	*NA*	—	—
Group 2 - 500 ppm IPRO								
Males	*NSA*	—	—	—	—	—	*CBE*	*NA*
Females	*42.83 ± 5.31**	*NA*	*1.89 ± 0.39*	*NA*	—	—	—	—
Group 3 – 5000 ppm IPRO								
Males	*NSA*	—	—	—	—	—	*CBE*	*NA*
Females	*44.91 ± 5.34**	*80*	*1.80 ± 0.53*	*90*	—	—	—	—
Group 4 – 20000 ppm IPRO								
Males	*NSA*	—	—	—	—	—	*CBE*	*28-38*
Females	*56.25 ± 4.67**	*80-90*	*1.96 ± 0.39*	*80*	*39.89 ± 1.42**	*50-80*	—	—

A, Measured by proliferating cell nuclear antigen (PCNA) immunohistochemistry; b, Iprovalicarb; c, the RF range within the observed neoplasms; NSA, not sex appropriate; NA, not applicable; CBE, cannot be evaluated, being PCNA negative; —, tissue was not utilized, * p<0.05.

Table 5.5.4.6-2. Pertinent Malignant and Benign Rat Neoplasms

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 5.5.4.6-2. Pertinent Malignant and Benign Rat Neoplasms (continued)

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 5.5.4.6- 3. Neoplasms and Early[a] 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.			

III. DISCUSSION

In male rats treated with lower (~20%) doses of iprovalicarb compared to females, at 20000 ppm, 3 presented with osteosarcomas (Table 5.5.4.6-2). 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 (Appendix 1 from study report). These neoplasms occurred only in high dose males, and not in high dose females, which received 20% higher dosing exposure of iprovalicarb for 2 years. A plausible explanation is that these osteosarcomas represent background neoplasms which are extremely rare and which occur at variable intervals, some around one year and some later. This rationale also may be 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 ([redacted], 1994; [redacted], 1998; [redacted], 1999; RITA, 1999; [redacted], 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) ([redacted] et al, 2002; [redacted] et al, 2005). These osteosarcomas were associated with extensive clinical and morphometric changes throughout the 2 year study, which bear no resemblance to the present findings.

The occurrence of malignant uterine neoplasms, especially the rare mixed Müllerian neoplasms, was associated with the mid dose (5000 ppm, 1/48) and high dose (20000 ppm, 2/50) of iprovalicarb,

all 3 cases observed at the terminal sacrifice (Table 2). 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 ([redacted], 1990) and metastasizes and displaces neighboring tissue and organs such as intestines and UB. In humans these tumors occur in younger patients ([redacted]on 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. Thus, the mean group RF of the non neoplastic tissue of all 3 dose levels was significantly increased (Table 5.5.4.6-1). Yet, these neoplasms occurred only in groups 3 and 4. Therefore, in all probability, these neoplasms reflect background neoplasms which occur later in life at low incidence, and are indirectly censored and diagnosed as sarcomas because of their anaplastic morphology. The relevance of these tumors to human Müllerian neoplasms is questionable (Kaspareit-Rittinghausen and Deerberg, 1990).

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; [redacted], 2003). The group RF mean of the nonneoplatic follicular thyroid tissue was comparable across all study groups (Table 5.5.4.6-1). Even the individual RF value of nonneoplastic areas was comparable to control values (Table 5.5.4.6-1), 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) (Appendix 1 from study report). Thus, all the follicular neoplasms are considered to reflect background neoplasms rather than IPRO-induced neoplasms.

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 5.5.4.6-2). The individual RF of each of these neoplasms was high both within and outside the neoplasm (Appendix 1 from study report). In addition, the group mean RF was significantly increased compared to controls (Table 5.5.4.6-1). This increase in RF cannot be explained directly, but it may be associated with iprovalicarb which is excreted in the urine. Nevertheless, the relevance to humans of these 2 benign neoplasms is questionable because there are major intraluminal differences between rats and humans. Thus, it is unlikely that the production of bladder neoplasia in rats by a nongenotoxic mode of action would be predictive of cancer hazard to humans ([redacted] et al, 1994; [redacted] et al, 1996; [redacted], 1998; [redacted] et al, 1999, [redacted] et al, 2004).

Finally, in contrast to the increases in neoplasia in the 4 tissues described and discussed above, there was a very significant decrease in a major endocrine responsive tissue of importance for human relevance, namely in the mammary gland (MG) in females. The incidence of MG adenocarcinoma was 6/50 in controls, 3/49 in low dose, 2/48 in mid dose and 0/50 in high dose females. This is a background neoplasm which occurs late in life ([redacted] and [redacted], 2004). Thus, this reduction indicates that IPRO possesses a propensity to down regulate endocrine (epigenetic) modifiers operational in this tissue and, by extension, in uterine tissues.

IV CONCLUSION

Iprovalicarb is not genotoxic and its structure does not contain any structural alerts. There was no evidence of iprovalicarb-induced carcinogenicity in male mice (up to 1567 mg/kg/day) or in female mice (up to 2544 mg/kg/day). Iprovalicarb did not induce carcinogenicity in male rats (up to 1110 mg/kg/day) or female rats (up to 1380 mg/kg/day). The appearance of increases at high doses of osteosarcomas/osteochondromas in males and Müllerian uterine, urinaru bladder papillomas and

thyroid follicular neoplasms in females is not causally related to iprovalicarb. The iprovalicarb-associated neoplasms in rats are not relevant for humans.

Report:	*[redacted];[redacted];2012;M-422060-01*
Title:	*Expert panel consensus report in assessing the weight of evidence of iprovalicarb carcinogenicity in rats and mice.*
Report No:	*010512*
Document No:	*M-422060-01-1*
Guidelines:	*Not specified*
GLP/GEP:	*no*

Executive summary

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, 5000, or 20000 ppm (equivalent to 0, 26, 263, or 1110 mg/kg/day in males and 0, 32, 326, or 1380 mg/kg/day in females). This study, entitled "SZX0722: Chronic toxicity and carcinogenicity investigations in Wistar rats (administration in the feed over 24 months)" was reported on February 4, 1998 ([redacted], 1998, M -001236-01-1, see section 5.5.2).

In order to monitor the rate of proliferation in certain target tissues, proliferating cell nuclear antigen (PCNA) immunohistochemical stain was performed on selected tissues from archival material (blocks) from the 24 month sacrifices. The PCNA results have been reported and the weight-of-evidence of all of the significant rat neoplastic findings has been assessed (see document M-267627-01-1). This expert panel consensus report confirms that the neoplasms observed in rats following a 2-year treatment-period with iprovalicarb are not relevant for humans. In conclusion, iprovalicarb is clearly considered as a non-carcinogenic compound.

KIIA 5.6 - Reproductive toxicity

Summary of reproductive toxicity studies

Table 5.6-1: Summary of reproductive toxicity studies

Type of study	Species	Dose range	NOAEL (mg/kg/day)	Reference
2-generation, oral	rat	0-100-2000-20000 ppm	parental: 2000 ppm ***(146.3)*** reproduction: 2000 ppm ***(146.3)***	[redacted], 1997; 26391
teratogenicity, oral	rat	0-100, 300, 1000 mg/kg/day	maternal: 1000 mg/kg bw/day developmental: 1000 mg/kg bw/day	[redacted], 1996; 24668
teratogenicity, oral	rabbit	0-100-300-1000 mg/kg bw/day	maternal: 1000 mg/kg bw/day developmental: 1000 mg/kg bw/day	[redacted], 1995; 24179

Iprovalicarb is not a reproductive toxin. No specific influence on either the reproduction performance of parents or the development of the young was detected. The lowest relevant reproduction NOAEL was 2,000 ppm (146.3 mg/kg bw/d) based on reduced pup weight at parentally toxic doses. The developmental NOAEL in both rat and rabbit was greater than 1,000 mg/ kg bw/d; No effects were seen up to and including the limit dose.

No further reproductive toxicity studies on iprovalicarb were required or generated.

Please refer to point IIA 5.6 (EU point 5.6) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.6.1 - Two generation reproductive toxicity in the rat

Report: [redacted]; [redacted]; 1997;M-001052-01
Title: SZX 0722 - Two-generation study in Wistar-rats
Report No: 26391
Document No: M-001052-01-1
Guidelines: US-EPA §83-4; OECD 416; JMAFF (1985);none
GLP/GEP: yes

KIIA 5.6.2 - Separate male and female studies

Not required according to Directive 91/414/EEC.

KIIA 5.6.3 - Three segment designs

Not required according to Directive 91/414/EEC.

KIIA 5.6.4 - Dominant lethal assay for the male fertility

Not required according to Directive 91/414/EEC.

KIIA 5.6.5 - Cross-matings of treated males with untreated females and vice versa

Not required according to Directive 91/414/EEC.

KIIA 5.6.6 - Effects on spermatogenesis

Not required according to Directive 91/414/EEC.

KIIA 5.6.7 - Effects on oogenesis

Not required according to Directive 91/414/EEC.

KIIA 5.6.8 - Sperm motility, mobility and morphology

Not required according to Directive 91/414/EEC.

KIIA 5.6.9 - Investigation of hormonal activity

Not required according to Directive 91/414/EEC.

KIIA 5.6.10 - Teratogenicity test by the oral route in the rat

Report: [redacted];[redacted];1996;M-000444-01
Title: SZX 0722 - Developmental toxicity study in rats after oral administration
Report No: 24668
Document No: M-000444-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

KIIA 5.6.11 - Teratogenicity test by the oral route in the rabbit

Report: [redacted];[redacted];1995;M-000290-01
Title: SZX 0722 - Developmental toxicity study in rabbits after oral administration
Report No: 24179
Document No: M-000290-01-1
Guidelines: **Deviation not specified**
GLP/GEP: **yes**

KIIA 5.7 - Neurotoxicity

Neurotoxicity was not observed in rats under acute up to 2,000 mg/kg and sub-chronic (90-day) exposure up to 20,000 ppm and more generally in the entire toxicology data package. A delayed neurotoxicity was not required.

Please refer to point IIA 5.7 (EU point 5.7) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.7.1 - Acute neurotoxicity - rat

Report:	[redacted];[redacted];[redacted];1997;M-000394-01
Title:	SZX 0722 - Acute oral neurotoxicity screening study in Wistar rats
Report No:	26021
Document No:	M-000394-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.7.2 - Delayed neurotoxicity following acute exposure

As iprovalicarb is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, testing is not necessary.

KIIA 5.7.3 - 28-day delayed neurotoxicity

As iprovalicarb is a fungicide with a completely different molecular structure than the known delayed-neurotoxic substances, testing is not necessary.

KIIA 5.7.4 - Subchronic neurotoxicity - rat - 90-day

Report:	[redacted];[redacted];[redacted];1997;M-001098-01
Title:	SZX 0722 (common name (proposed): Fencaramid) - Subchronic neurotoxicity screening study in Wistar rats (thirteen-week administration in the diet)
Report No:	26474
Document No:	M-001098-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.7.5 - Postnatal developmental neurotoxicity

Not required according to Directive 91/414/EEC.

KIIA 5.8 - Toxicity studies on metabolites

An acute oral rat toxicity study, a rabbit skin irritation, a skin sensitization assay (Magnus[redacted]& Kligmann) and an Ames test have been carried out with a plant metabolite of iprovalicarb, p-methyl-phenethylamine (M10 PMPA). These studies showed that PMPA is more acutely toxic than the parent compound (LD_{50} range from 300-500 mg/kg). It is corrosive to the rabbit skin but did not show any skin sensitizing potential in the guinea pig. It is also devoid of any mutagenic potential in bacteria. This metabolite was found in rat ADME studies. Therefore its toxicity profile is covered by the studies performed with the parent compound and thus considered as no toxicologically relevant.

Please refer to point IIA 5.8 (EU point 5.8) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

Report:	[redacted];[redacted];1996;M-000505-01
Title:	p-Methyl-phenethylamine (Metabolite of SZX 0722) - Study for acute oral toxicity in rats
Report No:	25319
Document No:	M-000505-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];[redacted];1996;M-000423-01
Title:	Acute skin irritation test (patch test) of alpha,4-dimethyl-benzenemethanamine in rabbits
Report No:	R6646
Document No:	M-000423-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];1996;M-000483-01
Title:	alpha-4-Dimethylbenzylamine - Study for the skin sensitization effect in guinea pigs (guinea pig maximization test according Magnusson and Kligman)
Report No:	25648
Document No:	M-000483-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

Report:	[redacted];1996;M-000513-01
Title:	p-Methyl-phenethylamine - Salmonella microsome test plate incorporation and preincubation method
Report No:	24894
Document No:	M-000513-01-1
Guidelines:	**Deviation not specified**
GLP/GEP:	**yes**

KIIA 5.9 - Medical and clinical data

Please refer to point IIA 5.9 (EU point 5.9) of the EU dossier submitted in the context of Annex I listing and the relevant data submitted during the EU evaluation process according to the "Review Report for Iprovalicarb (SANCO/2034/2000-FINAL, from July, 2002)".

KIIA 5.9.1 - Report on medical surveillance on manufacturing plant personnel

No data are available.

KIIA 5.9.2 - Report on clinical cases and poisoning incidents

With regard to iprovalicarb no cases of human poisoning have been reported up to mid of January 2012.

KIIA 5.9.3 - Observations on general population exposure & epidemiological studies

Up to now there is no known exposure of the general population to iprovalicarb. No epidemiological studies have been performed on iprovalicarb.

KIIA 5.9.4 - Clinical signs and symptoms of poisoning and details of clinical tests

Compound-specific poisoning signs in man after oral ingestion are not expected. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning.

KIIA 5.9.5 - First aid measures

- Remove patient from exposure / terminate exposure

- Thorough skin deciontamination with copious amounts of water and soap, if available with polyethylenglycol 300 followed by water.

- Flushing of the eyes with lukewarm water for 15 minutes.

- Induction of vomiting does not seem to be required in regard of the low toxicity.
It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious.

KIIA 5.9.6 - Therapeutic regimes

- As there is no antidote available for iprovalicarb, treatment has to be symptomatic and supportive.

- Gastric lavage does not seem to be required in regard of the low toxicity of the compound.

- The application of activated charcoal and sodium sulphate (or other carthartic) might be considered in significant ingestions.

KIIA 5.9.7 - Expected effects & duration of poisoning as a function of exposure

No cases of human poisoning with iprovalicarb are known. In an acute acute oral toxicity study in rats the limit dose was tolerated by the animals without any clinical signs or symptoms. After dermal application of 5000 mg/kg bw iprovalicarb to rats no systemic nor local signs were noted. This fits to the overall very low acute oral toxicity of iprovalicarb. Due to these facts poisoning via dermal exposure with iprovalicarb is also not expected in man. This applies also for inhalative exposure, since acute inhalation of 4977 mg iprovalicarb/m³ as dust for 4 hours was tolerated by rats without any clinical signs and symptoms.

KIIA 5.9.8 - Effects & duration of poisoning as a function of time

No cases of human poisoning with iprovalicarb are known and in most of the acute toxicity studies the limit dose was tolerated by the animals without any clinical signs or symptoms.

KIIA 5.9.9 - Dermal penetration

Please refer to thetier 2 summary, Annex III, section 3, point 7 (IIIA1.7.6) The dermal penetration studies on iprovalicarb (IPV) were carried out after the first inclusion of iprovalicarb in which a

10% default value was used. These studies were realized in 2003 using a WG formulation (84 g IPV/kg) comparable to the current representative WG formulation (90 g IPV/kg). Two dose levels were tested: a concentrate formulation (9.43 g IPV/L formulation) and a diluted formulation corresponding to the application phase (0.12 g IPV/L formulation). Taking into account the fact that generally the higher the dilution the higher the dermal penetration, these concentrations cover those encountered with the GAPs of the new representative formulation iprovalicarb + folpet WG 65.3 (granulate at 90 g IPV/kg for mixing loading and a diluted spray at 216 g IPV/400 L water = 0.54 g IPV/L).

For folpet the notifier refers to EFSA endpoints (EFSA Scientific Report (2006) 70, 1-78, Conclusion of the peer review of folpet).

KIIA 5.10 - Other/special studies

KIIA 5.11 - Summary of mammalian toxicity and overall evaluation

Similar to the whole dossier also this summary on mammalian toxicity and overall evaluation consists of old paragraphs, which were originally submitted for Annex I inclusion, and new parts. For discrimination the new parts are written in bold italic letters. The old paragraphs were adopted unchanged as far as possible. In areas in which the assessment changed in the old text, the changed wording is also printed in bold italic letters.

Toxicokinetics and Metabolism:

Iprovalicarb is readily absorbed from the GIT, and rapidly excreted. On average, more than or equal to 98% of the recovered radioactivity was excreted via urine and faeces within 48 hours. The major route of elimination was faecal for male rats and about equally faecal and renal for females. Metabolism was extensive, with >80% of the administered dose being metabolised within 48 hours. There was no evidence of bio-accumulation, with the intensity of radioactivity in almost all organs and tissues (except GIT, liver and renal cortex) being below the limit of detection after 8 hours. ≤0.5% of the oral dose remained in the body (excluding the GIT) at sacrifice (48 and/or 72 hours post-dosing). Only the liver retained a measurable amount of the dose at sacrifice, reaching 0.2% of the dose at most. The primary metabolite was the diastereomer pair of Iprovalicarb-carboxylic acid. The isomer ratio SS:SR was shifted in favour of the SR isomer in the urine of rats which received the high dose (20000 ppm) in the additional subchronic study.

Acute Toxicity:

The compound has a very low acute toxicity when tested orally, dermally or by the inhalation route. It is not irritant to the skin or eyes, and has no skin sensitising properties.

Short-term Toxicity:

After repeated oral administration of high doses of SZX 0722, no evidence of cumulative toxicity was seen in rats, mice or dogs. A daily dose of approximately 1980 mg/kg bw was tolerated in a 4-week feeding study by rats without increased mortality. In dogs, survival rates were unaffected up to and including approximately 1322 mg/kg bw over a 28 day treatment period.

In all three species investigated, the liver was identified as the main target organ, and the dog was the most sensitive species.

In the 13-week dog study, microsomal liver enzyme induction was observed at 250 ppm, the lowest dietary concentration tested (9.1 mg/kg bw/day), accompanied by elevated liver weights, macroscopic (discoloration, distinct lobulation) and microscopic (increased incidence of cytoplasmic changes) findings which are considered to represent (albeit minor) adverse effects. At higher doses, additional effects occurred that are expressions of a moderate to marked liver toxicity: elevated serum liver enzymes (AP as the most sensitive parameter), decreased protein levels, and histopathological alterations like hepatocellular hypertrophy, cytoplasmic vacuolation, focal necroses, single cell necroses, iron-containing pigments in hepatocytes and Kupffer cells, multilamellar bodies, and granulocytic infiltration. The gallbladder was also affected at the high dose of 50000 ppm: the abnormal contents seen at necropsy correlated microscopically with oedemas and dilation of the lymphatic vessels. Dogs in the two high doses were in a poor physical condition and a poor nutritional state; one animal had to be killed in a moribund condition. Secondary effects resulting from significant body weight losses were noted at necropsy and during histopathological evaluation such as atrophy of the fatty tissue of the subcutis and tongue or retarded growth of male sexual organs. A NOAEL could not be established, based on liver changes observed at the lowest dietary concentration level.

In the 13-week rat study, there was a slight indication of a treatment-related effect on liver function beginning at 5000 ppm (373 mg/kg bw/day) which was associated with increased liver weights. The effects were all small and there were no histopathological findings to back them up. At higher doses, AP-activities were elevated and pale livers were noted at necropsy. Indications were of effects produced by a weak inducer of microsomal enzymes with attendant slight effects on lipid metabolism. Results from the recovery groups have shown the reversibility of the liver effects after cessation of compound administration.

Effects on the mouse after dietary exposure to 7000 ppm (1724 mg/kg bw) led to liver changes which were adaptive responses to increased metabolism (increased relative liver weights and slightly increased cholesterol levels). There were no macroscopic or histopathological liver changes observed. At the highest dose of 14000 ppm, lower kidney weights in males and a trend to alterations in red blood count parameters were noted. However, no histopathological findings in support were observed.

Iprovalicarb was administered to dogs via the diet over 53 weeks. While no adverse effects were observed in females at the lowest dose administered (80 ppm, 2.62 mg/kg bw/day), the liver effects observed in males (increased ALAT and AP serum levels, microsomal enzyme induction and markedly increased liver weight in 2 of 4 dogs) corresponded to the more severe effects observed at higher dose levels. Therefore, an overall NOAEL could not be established. Marked hepatotoxicity was found at 800 ppm and above: elevated ASAT, ALAT and AP activities, cellular hypertrophy, periportal fatty changes and iron storage changes. At 8000 ppm, the GLDH and GGT activities were also increased, the plasma albumin levels depressed and there was an increase in both the absolute and relative liver weights. The liver size was enlarged, and obvious lobulation and discolouration was observed at necropsy. Focal necroses, an increased incidence of bi-nucleated hepatocytes were seen (in addition to the changes seen at 800 ppm) during the histopathological examination. As was apparent in the 13-week study, effects on the gallbladder were also noted (adhesive mucus, formation of pseudoglands in the gallbladder wall) at 800 ppm and above. A slight increase in

poikilocytosis was seen in females at 800 ppm and above, and in high dose (8000 ppm) males. A marginal increase in normoblasts occurred in high dose females.

Hence, a NOAEL could not be established from the 53-week dog study, as increased liver weight and liver enzyme induction occurred at all dose levels tested. A supplemental 4-week study was carried out to investigate liver enzyme induction in a lower dose range, and to obtain a NOEL with regard to N-demethylase, O-demethylase and cytochrome P450 specifically. This data provided us with a NOEL of 20 ppm (0.77 mg/kg bw/day for males/females). The 80 ppm data from both the chronic study and the supplementary study were in agreement. No cumulative effect was seen, and the effects were seen to be reversible. In contrast to the 53-week dog study, there were no increases in serum AP or ALAT activities and no liver weight increases after 4 weeks exposure at dose levels up to 80 ppm, indicating that prolonged treatment is necessary to provoke such effects.

In a subacute inhalation toxicity study on rats, the maximum technically feasible concentration of 504.4 mg/m^3 air was tolerated without any compound-related effect on the parameters investigated.

In a subacute dermal toxicity study, rabbits receiving the limit dose of 1000 mg/kg bw did not reveal any local skin findings or signs of systemic toxicity.

Genotoxicity:

The genotoxic potential of Iprovalicarb was investigated in bacteria and mammalian cells using various *in vitro* and *in vivo* assays. None of the tests revealed any evidence of mutagenic or genotoxic activity. The substance did not induce point mutations, DNA damage or chromosomal aberrations. In addition, an organ-specific ^{32}P-postlabelling assay *in vivo* in the uterus and urinary bladder epithelium of female rats was conducted to investigate DNA-adduct formation by Iprovalicarb treatment. No genotoxic potential identified for this substance in this study either.

Long-term toxicity and carcinogenicity:

Long term studies were conducted with rats and mice. In mice, administration of Iprovalicarb over 105 weeks resulted in slight increases in body weight-related food and water intakes in males of the high dose group (7000 ppm), while body weight was slightly depressed. There was slightly elevated blood urea level in both sexes at 1400 ppm and above, indicating a minor reduction in kidney function, which would explain the observed increases in water intake of high-dose animals. 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 the males of these dose groups. No such effect was seen in any of the females.

Elevated triglyceride levels and an associated increase in the incidence of 'cellular fatty changes' in the livers of high dose males was observed. However, more detailed descriptions of the lesions point more likely to these being degenerative cell changes.

In the chronic rat study, the effects on body weights in males at 20000 ppm and females at 5000 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.

Changes in liver morphology and function, beginning at 5000 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.

There was an increase in malignant mixed Muellerian tumours in the uteri of females treated at 5000 and 20000 ppm (1/50 and 2/50, respectively, as against 0/50 in controls). No documented cases of the induction of such tumours have been found in the literature. As a spontaneous occurrence, the finding was reported for the first time in 1990. Spontaneous incidences per study varied between 0% and 2%. However, in a supplementary ^{32}P-postlabelling assay performed with uterine tissue after treatment of rats with Iprovalicarb gave no evidence of a DNA-adduct forming potential of the test substance ([redacted]., 1998).

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 20000 ppm, squamous cell carcinoma of the clitoral gland was observed. Clitoral glands are not routinely investigated, but only when some macroscopic changes are visible. Historical data are also rare and for these reasons, it is difficult to assess the toxicological significance of this finding. However, it should be noted that this was only seen at the very high dose of 20000 ppm.

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 slightly above the range of historical values, which could indicate a treatment-related effect. However, in a supplementary ^{32}P-postlabelling assay performed with urinary bladder epithelium after treatment of rats with Iprovalicarb gave no evidence of a DNA-adduct forming potential of the test substance ([redacted]., 1998). Therefore, there is no evidence of a primary carcinogenic potential of the test substance.

Malignant tumours of the skeletal system were seen in 20000 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.

There was a statistically significant positive trend in the incidence of follicular cell adenomas (5000 and 20000 ppm groups). 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. Additionally, incidences of the follicular cell neoplasms are covered by the range of historical data and can therefore be treated as spontaneous events. No effect was seen in the thyroids of males.

Curiously, in high dose group animals, slightly decreased incidences of non-neoplastic and/or tumour lesions were found for the female mammary gland (decreased diffuse hyperplasia, adenocarcinoma) and for the pituitary gland (decreased hyperplasia of the pars distalis).

In conclusion, the histopathological findings indicate a shift in the incidences of certain tumours at 5000 ppm (mixed Muellerian uterine tumours), and at 20000 ppm (squamous cell carcinomas of the clitoral gland, 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. However, the increased incidence was only minimal and close to the historical control range. In view of the decreased incidences of mammary and pituitary gland lesions in combination with increased incidences of urogenital tumours in females of the high-dose group, it appears possible that the tumour induction is preceded by disruption of hormone regulation at high dose levels.

It should be noted that the animals concerned were all subjected to extremely high doses (20000 ppm, 1110 mg/kg bw/day) , as the test substance was observed to be of very low general toxicity in subchronic studies.

Furthermore, neither the battery of genotoxicity tests nor the supplementary ^{32}P-postlabelling assay revealed a genotoxic potential of the test substance. It seems unlikely, therefore, that Iprovalicarb poses any threat of a carcinogenic potential.

The tumourigenic NOEL for females is 500 ppm (26 mg/kg bw/day), based on the occurrence of mixed Muellerian tumours in the uterus at 5000 and 20000 ppm, and carcinomas of the clitoral gland, and papillomas of the urinary bladder at 20000 ppm only. The tumourigenic NOEL for males is 5000 ppm (262.5 mg/kg bw/day), based on the occurrence of malignant tumours of the skeletal system at 20000 ppm.

The PCNA evaluation of the tissues selected from the 2-year rat carcinogenicity study showed that iprocalicarb did not induce a biologically relevant increase of PCNA positive cells in selected tissue of neoplasms.

In conjunction with the information gleaned from the supplementary studies (special metabolism study and the associated biokinetics study, as well as a bioavailability study, the ^{32}P-postlabelling in vivo assay, and the liver foci test), which were carried out to elucidate the findings in the chronic rat study, the following can be concluded: SZX 0722 does not possess a carcinogenic potential.

Reproductive Toxicity:

The reproductive toxicity of SZX 0722 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. A possible slight decrease of the female/male ratio was observed for both the F1 and F2 offspring of the 20000 ppm group. At the highest dose also, pup body weights during the lactation phase were reduced in both the F1 and F2 generations, slightly reduced mean litter weight at birth and at weaning (day 28) in F1 pups, an increase in relative liver weights in F2 weanlings, and a reduced lactation index in F1 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. A NOAEL of 2000 ppm was established for both parental and reproductive toxicity.

In both species tested, Iprovalicarb, up to and including the highest dose level (1000 mg/kg b.w.), 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 b.w..

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

Neurotoxicity:

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

In the subchronic (13-week) neurotoxicity study, evidence of slight, dose-related, non-specific general toxicity was noted. The only neurobehavioural effect observed was a marginal decrease in the motor activity measurements in females of the highest dose group during study week 8; no difference was observed between groups at the 13-week time-point. However, this effect is not regarded to be indicative of a neurotoxic potential. 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 NOEL with respect to neurotoxic potential.

In conclusion, SZX 0722 does not have a neurotoxic potential, after either acute or subchronic administration.

Further toxicological studies:

Acute toxicity studies were conducted to test p-Methyl-phenethylamine (PMPA), a metabolite of Iprovalicarb. The oral LD_{50} (200-2000 mg/kg) was lower than that of the parent compound, it was corrosive to the skin but was not sensitising to the skin in the Guinea Pig Maximisation Test (Magnus [redacted] and Kligman). PMPA showed no genotoxic potential in the Ames test.

Medical data:

No data are available on medical surveillance on manufacturing plant personnel. During intensive experimental biological testing and field use of formulations containing SZX 0722, no negative experiences affecting the health of workers were reported. Compound-specific poisoning signs in man after oral ingestion are not expected. The analytical demonstration of parent compound or metabolites in blood, urine or gastrointestinal contents is required for an exact diagnosis of poisoning. No specific antidotal therapy is available for the treatment of oral poisoning. Any contamination of the skin should be washed off immediately with plenty of water. The removal of ingested compound by (preferably) gastric lavage or induction of vomiting followed by symptomatic treatment is recommended in cases of oral uptake of SZX 0722. The effects in man following oral uptake of toxic doses of SZX 0722 are not known.

Table 5.11.1 NOAELs and findings at LOAELs

Study/dose levels (ppm)	NOAEL (ppm)	NOAEL (mg/kg bw)	Findings at the LOAEL
28 day feeding study, Wistar rat 0, 2000, 6000, 20000	2000	195.8	Clinical chemistry, enzyme induction and increased liver weight at 6000 ppm
4-week feeding study, purebred beagle 0, 100, 1000, 10000, 50000	100	2.7	Hepatocytes with 'ground glass' appearance, subtle AP increase
13 week feeding study, Wistar rat 0, 1250, 5000, 20000	5000	372.7	Rel. liver weights increased by > 10%
13 week feeding study, B6C3F1 mouse 0, 280, 1400, 7000, 14000	1400	325	Slight ↑ in MCV and cholesterol in males and females, slight ↑ liver weight in females, slight ↑ water intake and ↓ kidney wt. in males
13 week feeding study, purebred beagle 0, 250, 2500, 50000	< 250 (NOAEL not established)	9.1 (NOAEL not established)	At 250 ppm: slight ↑ in abs. and rel. liver weight, liver enzyme induction, minimal hepatocellular cytoplasmic change. ↑ activity of AP at higher dose levels
53 week feeding study, purebred beagle 0, 80, 800, 8000	80 (for females only)	2.62	80 ppm (males): ↑ activity of serum ALAT and AP, ↑ liver wt,liver enzyme induction. 800 ppm and above: ↑ serum enzyme activities, ↑ liver wt, relevant histopathological findings
5 day inhalation study, Wistar rat 0, 20.6, 102.9, 504.4 mg/m³	≥ 504.4 mg/m³ (NOEC)	181	This was the max. technically feasible concentration
4 week subacute dermal toxicity study, HC:NZW rabbits 0, 1000 mg/kg bw/day		≥ 1000 (/α)	No effects observed at the limit dose
Combined chronic toxicity/ carcinogenicity study, Wistar rat 0, 500, 5000, 20000	500	26.0	5000 ppm and above (females): ↓ bw, clinical chemistry, ↑ rel. liver wt and ↑ hepatocellular hypertrophy. Occurrence of mixed Mullerian tumours 20000 ppm (males): Marginal ↓ bw, ↑ AP activity; occurrence of malignant skeletal tumours. 20000 ppm (females): carcinomas of the clitoral gland, and papillomas of the urinary bladder
Oncogenicity study, B6C3F1 0, 280, 1400, 7000	280	58.5	1400 ppm and above (both sexes): slightly ↑ blood urea levels, slightly ↓ kidney wt. 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.
Two generation reproduction, Wistar rat 0, 100, 2000, 20000	2000 (parents) 2000 (foetal)	146.3	F0: ↓ body weight and ↑ liver weight Offspring: ↓ body weight during lactation, ↓ mean litter weight, ↑ liver weights (F2), and ↓ lactation index in F1 pups
Developmental toxicity, Wistar rat (gavage) 0, 100, 300, 1000 mg/kg bw/day		≥ 1000	No treatment-related effects
Developmental toxicity, Russian rabbit (gavage) 0, 100, 300, 1000 mg/kg bw/day		≥ 1000	No treatment-related effects
Supplementary subacute dog feeding study 0, 10, 20, 40, 80	20	0.77	↑ N-demethylase activity, slight ↑ Cyt P450

Calculation of the acceptable daily intake (ADI)

Looking at the toxicological profile of SZX 0722, the risk to the consumer of treated crops with respect to acute toxicity, organotoxicity, genotoxicity, and carcinogenicity is not discernible. There is no evidence of bioaccumulation, the 2-generation reproduction study in rats and the oral developmental toxicity studies in rats and rabbits have shown that SZX 0722 has no primary reproductive toxic or developmental toxic potential.

The calculation of the acceptable daily intake is based on data from the chronic feeding studies in the dog and the rat, and the oncogenicity study in the mouse (Table 6.10.2.1).

In the case of SZX 0722, the dog has been shown to be the most sensitive species. Thus, the ADI can be calculated as follows:

A NOAEL of the chronic dog study was established at the lowest dose of 80 ppm (2.62 mg/kg bw/day) for females only. In males administered 80 ppm, findings of increased levels of serum AP activity, and microsomal enzyme induction were corroborated by substantially increased liver weights observed in two of the four dogs. In view of the increased severity of hepatotoxicity at higher doses, the effects observed at 80 ppm in males are regarded to be adverse. However, in the absence of histopathological changes and of adverse effects in low-dose females, and because reversibility of liver enzyme induction was demonstrated in a supplementary 4-week dog study, it appears likely that the dose level of 80 ppm is close to the NOAEL of the 53-week dog study. Therefore, using a safety factor of 200, the resulting ADI is 0.015 mg SZX 0722 bw/day.

Acceptable Operator Exposure Level (AOEL)

The NOAEL's observed in the subacute and subchronic toxicity studies performed in rats, mice and dogs and the developmental toxicity studies in rats and rabbits are used in order to set an AOEL. The NOAEL's and the effects seen at the lowest effect levels are summarised for the toxicological studies performed with SZX 0722.

It is considered appropriate to base the AOEL on NOAELs from studies which were conducted with dogs (most sensitive species). Since an unequivocal NOAEL could not be derived in the 13-week feeding study, (toxicologically relevant liver effects at the lowest dose of 250 ppm) and the 4-week range-finding study (NOAEL of 100 ppm) used only a limited number of dogs, the lowest relevant NOAEL/LOAEL is from the 53-week feeding study with dogs. Thus, the value of 2.62 mg/kg bw/day (80 ppm) is used for the calculation of the AOEL. As for the calculation of the ADI, an additional uncertainty factor of 2 is introduced to account for the slightly adverse effects observed in male dogs at this dose. Therefore by use of an overall assessment factor of 200, the AOEL is set at 0.015 mg/kg bw/day. Since intestinal absorption of Iprovalicarb is high, the oral AOEL is considered equivalent to the internal AOEL.

Acute Reference Dose (ARfD)

This is not relevant for SZX 0722, which is of low acute toxicity and poses no long-term risks from acute intake.