Document Title

Summary of the toxicological and metabolism studies for Deltamethrin

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013
Document MCA
Section 5: Toxicological and metabolism studies

According to the guidance document, SANCO 10181/2013, for preparing dossiers for the approval of a chemical active substance

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2015-10-16	CA 5.2- CA 5.8 updated concerning study summaries, classification and labelling	M-480332-02-1
2015-12-02	CA 5.2- CA 5.8 updates highlighted in yellow CA 5.1 and CA 5.1.1 updated concerning requested position papers on: - M-533554-02-1 and M-539732-01-1: more detailed information on the metabolic pathway of deltamethrin in rat and comparing this metabolism with those in plants, goats and the environment. - M-533554-02-1: an overall summary of the kinetic profile of deltamethrin. - M-291817-01-1: more quantitative details on the endpoint of oral absorption to be used in the adjustment of the AOEL	M-480332-03-1
2017-02-02	CA 5.4.1 inclusion of new studies M-577646-01-1 and M-577648-01-1 CA 5.8.1: inclusion of additional information M-559648-01-1 CA 5.8.1: inclusion of additional information M-559823-01-1	M-480332-04-1

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

Additions to the document after the Completeness Check are highlighted in yellow. Content not necessary anymore is crossed out.

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

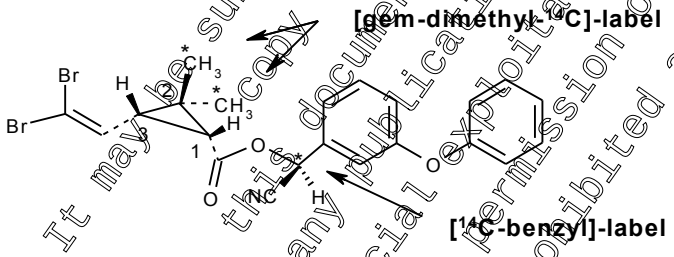
INTRODUCTION

This document contains only summaries of studies, which were not available at the time of the last Annex I inclusion of deltamethrin and were therefore not evaluated during the last EU review of this compound. A short summary of the toxicological endpoints from the last EU review has been provided and adapted with the new information where necessary. In order to facilitate discrimination between new and original information in tables, the references of new studies are mentioned in bold black in tables. All studies, which were already submitted by Bayer CropScience for the previous Annex I inclusion, are contained in the Monograph, its Addenda and in the original (baseline) dossier provided by Bayer CropScience and are not summarized in this document. References to these studies are written in grey in tables. Endpoints set with the last Annex I inclusion of deltamethrin are listed in the respective review report and are discussed at the end of this section.

CA 5.1 Studies on absorption, distribution, metabolism and excretion in mammals

The absorption, distribution, excretion and metabolism of deltamethrin in mammals were investigated in rats using ^{14}C-labelled deltamethrin. The chemical structure and different positions of the ^{14}C labelling used are given in the figure below. A single low dose, a single high dose and repeated low doses were administered orally to rats to investigate the distribution, excretion and metabolism of deltamethrin ([redacted]; 1990; M-149312-01-1). The gastrointestinal absorption following oral exposure was estimated by comparing the amount of radioactivity excreted after a single oral low dosing with that of a single intravenous low dosing ([redacted]; 1993; M-132447-01-1). The results of relevant studies is briefly summarised below.

Figure 5.1-1: Different ^{14}C-label positions of deltamethrin



Absorption:

The gastrointestinal absorption of deltamethrin in rats following oral exposure was estimated by comparing the ratio of radioactivity excreted in the urine and in the faeces following a single oral and a single intravenous dose, with the intravenous dose being 100 % systemically available and therefore representing a 100% absorption.

A gastrointestinal absorption of 75% in rats following oral dosing of deltamethrin was estimated by this comparison.

Distribution:

After oral administration, deltamethrin was distributed to most organs and tissues. Residues in organs, tissues and carcases were low with less than 2% of the dose 7 days after administration of a single oral low or high dose or repeated oral low doses. Following oral administration of deltamethrin, the highest radioactive residues were found in fat (0.07 µg equ/g after a low dose; 0.84 µg equ/g after a high dose; 0.09 µg equ/g after repeated low doses), 7 days after dose administration. The distribution of orally administered deltamethrin within the body was independent of the dose level or dosing regime (single or repeated dosing). No sex related differences were observed.
One hour after intravenous dosing of 2.4 mg deltamethrin/kg body weight, the blood level amounted to 3.01 µg equ/g. At this time point highest radioactive residues were found in the excretory organs liver and kidneys, as well as in ovaries and fat. Lowest radioactive residues, below 1 µg equ/g, were found in brain, muscles, spinal cord and sciatic nerve, which dropped to or below the LOQ at study end 120 h after intravenous dosing. At study end, all radioactive residues were below 0.08 µg equ/g, except for fat and ovaries. Elimination of radioactivity from blood, organs and tissues could be described by single first order kinetics. The elimination of deltamethrin was rapid from blood and most organs and tissues with elimination half-lives ranging from 4.8 – 7.1 hours. Only in fat, sciatic nerve and skin back region the elimination of deltamethrin was slower.

Excretion:

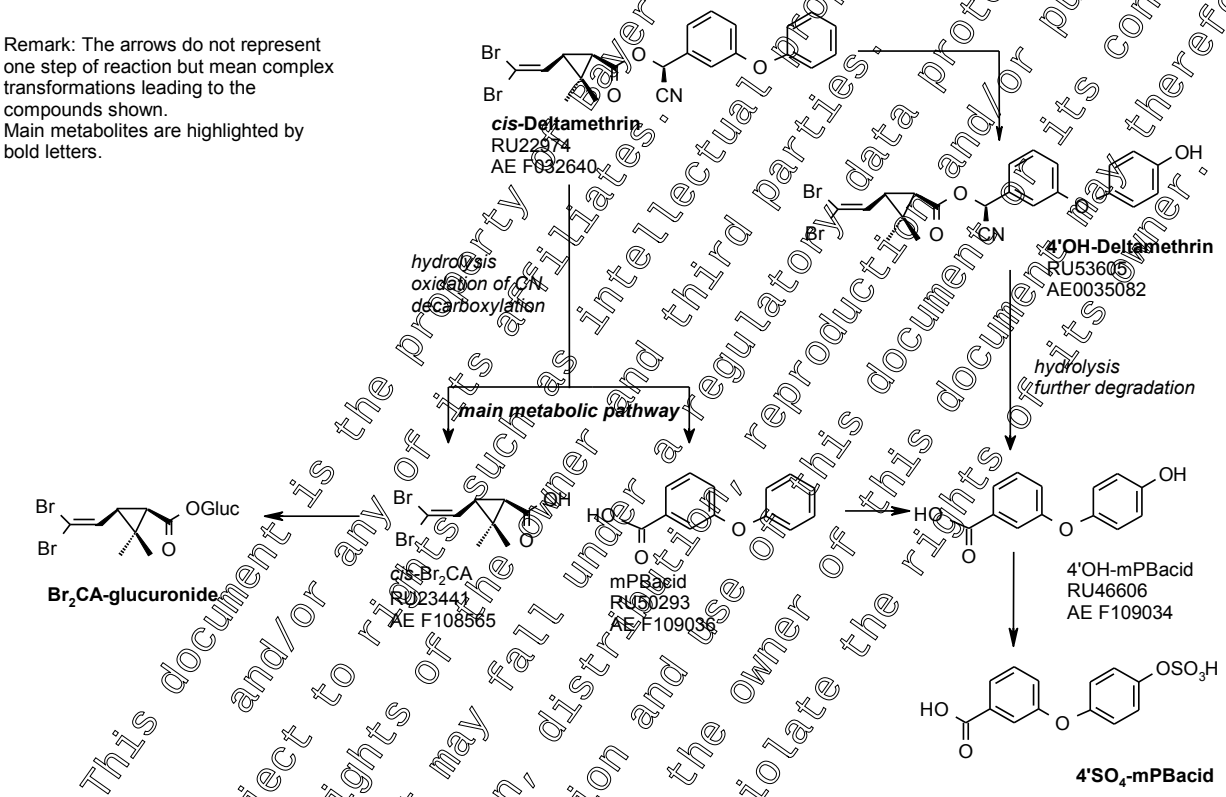
After a single oral low or high dose or repeated oral low doses, deltamethrin was excreted relatively fast and completely by rats. The majority of the administered single low or high dose (> 70%) or repeated doses (> 64%) was excreted within the first 24 hours after dosing. The recovery in excreta ranged from 74% to 91% of the administered radioactivity for the different dosing regimes.
After administration of a single oral low dose, slight ^{14}C-label related differences in the route of excretion were observed for male rats. For the ^{14}C-dimethyl label, male rats excreted slightly more radioactivity with the faeces, whereas for the ^{14}C-benzyl label the urinary excretion was slightly preferred. In female rats both excretion routes were pretty evenly split for either ^{14}C-label.
After repeated oral low doses, deltamethrin was mainly excreted via urine, except for male rats dosed with ^{14}C-dimethyl deltamethrin, which excreted slightly more radioactivity in faeces. After a single oral high dose, deltamethrin was excreted mainly via faeces. No sex or ^{14}C-label dependent differences in the route of excretion were observed.
After a single intravenous low dose, deltamethrin was excreted relatively fast and completely by female rats. More than 60% of the administered low dose was excreted within the first 24 hours after dosing. After intravenous dosing of ^{14}C-benzyl deltamethrin, the renal excretion was the preferred route. At study end, 120 hours after dosing, 60% of the administered dose was excreted in urine and 27% was excreted via the bile into the faeces.

Metabolism:

Deltamethrin was rapidly and extensively metabolised in rats. The main route of metabolism was the cleavage of the ester bond and hydroxylation on the 4' position of the alcohol moiety (not necessarily in that order). The cleavage of the ester bond is the important metabolic step in the detoxification of deltamethrin. A portion of the metabolites resulting from cleavage of the ester bond were conjugated before being excreted with the urine. Major metabolites identified in urine were 4'SO_4-mPBacid and mPBacid for the ^{14}C-benzyl-label and Br_2CA-glucuronide and Br_2CA for the ^{14}C-dimethyl label. No unchanged deltamethrin or metabolites containing the ester bond were observed in the urine although unchanged deltamethrin was a major compound in the faeces. In faeces 4'OH-deltamethrin was

identified with both ^{14}C-labels, beside 4'OH-mPBacid, which was detected with the ^{14}C-benzyl label. No other metabolites comprising greater than 10% of the dose administered were present in urine or the faecal samples analysed. No sex or dose related differences were observed in the metabolic pattern. Repeated dosing showed no influence on the metabolic pathway. The metabolic pathway in rats is given in **Figure 5.1-2**.

Figure 5.1-2 Metabolic pathway of deltamethrin in rats



Upon request by the RMS UK the notifier Bayer CropScience has prepared the two position papers M-533554-02-1 and M-539732-01-1 providing more detailed information on the metabolic pathway of deltamethrin in rat and comparing this metabolism with those in plants, goats and the environment. The document M-539732-01-1 also includes a table of all significant metabolites identified in the different compartments and their quantitative occurence. The documents M-479846-02-1 and M-328058-02-1 which are cited in the position paper M-539732-01-1 were also included in this dossier. Furthermore the position paper M-533554-02-1 provides an overall summary of the kinetic profile of deltamethrin.

CA 5.1.1 Absorption, distribution, metabolism and excretion by oral route

For already evaluated studies, please refer to MCA 5.1.

Report:	KCA 5.1.1/08; [redacted]; 2014; M-475952-01-1
Title:	[Benzyl-14C]deltamethrin: Metabolic stability and profiling in liver microsomes from rats, mice and humans for inter-species comparison
Report No.:	EnSa-13-0820
Document No.:	M-475952-01-1
Guideline(s):	Regulation (EC) No 1107/2009 (Europe) amended by the Commission Regulation Regulation (EC) No 283/2013 (Europe) US EPA OCSPP 870.SUPP
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

The comparative metabolism of ^{14}C-deltamethrin was investigated in *in-vitro* systems by incubating the test item with liver microsomes from male Wistar rats (RLM) and from humans (HLM) in the presence of NADPH cofactor.
The results of the tests demonstrated that extensive *in vitro* metabolism in both species occurred. Some differences in the vitro metabolic pattern between rat and human were observed. However, all human metabolites were also present in the rat. No metabolite specific to humans was observed. In incubations with rat liver microsomes, 49.4% of the initial ^{14}C-deltamethrin remained unchanged and the rest was metabolised towards 13 metabolites.
In human liver microsomes, although ^{14}C-deltamethrin was metabolised to a significantly lower number of metabolites, only 4.2% of ^{14}C-deltamethrin remained after 1 h incubation, indicating a fast metabolism rate of ^{14}C-deltamethrin in human liver microsomes.

The most abundant metabolites formed by human liver microsomes were also detected as major metabolites in incubations with rat liver microsomes.

The results suggest that in incubations with human liver microsomes no additional deltamethrin metabolites are formed than compared to incubations with rat liver microsomes.

Materials and Methods

Test System
Pooled liver microsomes from male Wistar rats (RLM) and humans (HLM) were incubated with [Benzyl-^{14}C]-deltamethrin in the presence of NADPH cofactor. The 15 µM test item concentration was chosen in order to have enough sample material for possible identification of metabolites by chromatographic or spectroscopic methods. The sampling times were 0 and 1 hour after test start. The test duration of 1 hour for the test item was considered as reasonable because positive results were obtained from the enzymatic reaction of testosterone to hydroxy-testosterone already after 10 minutes. Samples were analyzed following protein precipitation by reversed phase HPLC with radiochemical detection (HPLC-RAD).

Results

The recovery of radioactivity was measured in the microsome incubations and amounted to 96.0% in RLM and 105.0% in HLM for the 1-hour samples.
The metabolic activity of the microsomes was clearly demonstrated by determining 6β-hydroxytestosterone that was formed from testosterone by testosterone 6β-hydroxylase. This biochemical reaction is well known for the CYP3A microsomal enzyme.

In incubations with rat liver microsomes, 49.4% of the initial ^{14}C-deltamethrin remained unchanged. ^{14}C-Deltamethrin was metabolised extensively. Under the experimental conditions used in the present study, a total of 13 metabolites were detected, 5 of them were above the limit of quantification and 4 above 5% of the relative percentage.
In human liver microsomes, ^{14}C-deltamethrin was metabolised nearly completely but to a significantly lower number of metabolites. Remaining 14C-deltamethrin after 1 h incubation was only 4.2%, indicating a fast metabolism rate of ^{14}C-deltamethrin in human liver microsomes.

All metabolites found in the human microsomal incubations were also present in the rat microsomal incubation. The most important metabolites formed by human liver microsomes were also detected as major metabolites in incubations with rat liver microsomes.

Conclusion

Incubations of deltamethrin in rat and human liver microsomes showed that extensive in vitro metabolism in both species occurred. The metabolism of deltamethrin in the human microsomal incubation was enhanced and nearly complete compared to rat. This is potentially indicative for a more effective detoxification of deltamethrin in humans. All metabolites observed after incubation with human microsomes were also present after incubation with rat microsomes. No metabolite specific to humans was observed. In rat more metabolites than in human were observed under the tested conditions. These differences in the metabolism of deltamethrin between rat and humans may be due to intrinsic differences in activity of the different enzymes involved in the metabolism.
The assumption is supported by two publications (see below).

In the course of the literature search (please refer to MCA 9) Bayer CropScience came across two publications investigating the depletion of deltamethrin in human and rat microsomes. They are also describing quantitative differences after exposure of deltamethrin to isolated isoforms of human and rat specific cytochromes and carboxylesterases. Although these two publications do not change any end point or risk assessment, they are nevertheless summarised below as supportive data:

Report:	KCA 5.1.1/09; [redacted]; 2006; M-476902-01-1
Title:	Species differences in the in vitro metabolism of deltamethrin and esfenvalerate: differential oxidative and hydrolytic metabolism by humans and rats.
Report No.:	M-476902-01-1
Document No.:	M-476902-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

As part of the study, the elimination of two pyrethroids from rat and human liver microsomes were investigated. Parent depletion was measured in the presence and absence of NADPH to assess quantitative differences in biotransformation pathways, rates of elimination, and intrinsic hepaticclearance between rat and human microsomal incubations. Additionally, the hydrolyses of the two pyrethroids mediated by rat and humans carboxylesterases (CEs) were investigated.

No metabolic profiles were measured in the incubations. However, HPLC measurement of hydrolyses products like alcohols and acid metabolites were mentioned but not detailed in the publication.

The respective pyrethroid (1 µM) was incubated from 0 to 10 min in 1.5 ml of 0.1 M Tris containing 1.0 mg of MSP/ml and 1.0 mg of NADPH/ml. NADPH-independent assays were carried out from 0 to 30 min to ensure sufficient elimination to calculate elimination rates. Assays were carried out in duplicate in a shaking water bath at 37 °C and 250-µl aliquots were removed at each time point for liquid chromatography/tandem mass spectrometry analysis. Assays were repeated in the presence of 200 µM TEPP to inhibit esterase activity. A volume of 10 ml of 30 mM TEPP in methanol was added to the assay before incubating for 10 min at 37°C before the addition of the pyrethroid. Depletion of parent compound was measured via LC/MSD.
Hydrolysis of pyrethroids by rat and human CEs were performed at a single saturating concentration of pyrethroid (50 µM) to compare the hydrolysis rates of each enzyme (specific activity).

Deltamethrin was eliminated primarily *via* NADPH-dependent oxidative metabolism in rat liver microsomes. In human liver microsomes, deltamethrin was eliminated mainly via NADPH independent hydrolytic metabolism. The intrinsic hepatic clearance for deltamethrin was estimated to be 2-fold more rapid in humans than in rats on a per kilogram body weight basis.
Results of isolated rat and human carboxylesterases (CEs) metabolism experiments revealed that human carboxylesterase 1 (hCE-1) was markedly more active toward deltamethrin than the class 1 rat CEs hydrolase A and B and the class 2 human CE (hCE-2).

The study demonstrated a difference in the in vitro pathways of biotransformation of deltamethrin in rat and human liver microsomes, which is due in part to differences in the intrinsic activities of rat and human carboxylesterases.

Report:	KCA 5.1.1/10; [redacted]; 2007; M-458601-01-1
Title:	Identification of rat and human cytochrome P450 isoforms and a rat serum esterase that metabolize the pyrethroid insecticides deltamethrin and esfenvalerate.
Report No.:	M-458601-01-1
Document No.:	M-458601-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

The metabolism of the two pyrethroids esfenvalerate and deltamethrin by rat and human liver microsomes differs with respect to the biotransformation pathway (oxidation *versus* hydrolysis) responsible for their clearance as described in the previous publication. This study further explored the species differences in the metabolism of these chemicals. Using a parent depletion approach, rat and human cytochromes P450 (P450s) were screened for their ability to eliminate the two pyrethroids during in vitro incubations. Rat P450 isoforms CYP1A1, CYP2C6, CYP2C11, and CYP3A2 and human P450 isoforms CYP2C8, CYP2C19, and CYP3A5 were capable of metabolising either pyrethroid. Human CYP2C9 did not metabolise deltamethrin. Rat and human P450s that metabolised both pyrethroids do so with similar kinetics.
In addition to the liver, a potential site of metabolic elimination of pyrethroids is the blood *via* serum carboxylesterase (CE) hydrolysis. The serum of rats, but not humans, contains significant quantities of

CE. The investigated pyrethroids were metabolised effectively by rat serum and a purified rat serum CE. In contrast, neither pyrethroid was metabolised by human serum or purified human serum esterases (acetylcholinesterase and butyrylcholinesterase). These studies suggest that the difference in rates of oxidative metabolism of pyrethroids by rat and human hepatic microsomes is dependent on the expression levels of individual P450 isoforms rather than their specific activity.

Upon request by the RMS UK the notifier Bayer CropScience has provided with document M-291817-01-1 (cited in position paper M-533554-02-1) more quantitative details on the endpoint of oral absorption to be used in the adjustment of the AOEL. (Relevant metabolites are considered in case necessary.)

CA 5.1.2 Absorption, distribution, metabolism and excretion by other routes

Please refer to MCA 5.1.

CA 5.2 Acute toxicity

The acute oral toxicity of deltamethrin displays significant differences depending on the solvent used for the administration. When the administration was done in corn oil to not fasted Sprague Dawley rats, the LD_{50} was 95 mg/kg in males (95% confidence limits: 74 – 122 mg/kg) and 87 mg/kg in females (95% confidence limits: 77 – 97 mg/kg) ([redacted]; 1996; M-139700-01-1). In a recent study performed at the request of Brazilian authorities, no mortalities were observed at 2000 mg/kg in Wistar rats when deltamethrin was dissolved in 0.5% aqueous carboxymethylcellulose sodium ([redacted]; 2005; M-263224-01-1). Based on the LD_{50} obtained in corn oil, deltamethrin is labelled Toxic R25 or GHS category 3 H301 (Toxic if swallowed).

Deltamethrin is not toxic through the dermal route with an LD_{50} > 2000 mg/kg. This result was confirmed in a recent study performed by [redacted] in 2005 (M-258954-01-1) at the request of Brazilian authorities.

No new acute inhalation study was performed. The LC_{50} taken into consideration is 0.6 mg/L obtained by [redacted], in 1978 (M-101619-01-1). Based on these results, deltamethrin is labelled Toxic R23 or GHS category 3 H331 (Toxic if inhaled).

Deltamethrin is not irritant to the skin or the eyes and is not a skin sensitizer (Magnusson and Kligman or Buehler tests), as confirmed by new studies ([redacted]; 2005; M-260123-01-1, [redacted]; 2005; M-260858-01-1 and [redacted]; 2005; M-261562-01-1, Buehler patch test).

Due to the new data requirements a photoxicity study is required if the molar extinction coefficient is higher than 10 L x mol^{-1} x cm^{-1}. This is the case for deltamethrin so a photoxicity study has been conducted and this shows that deltamethrin does not possess any phototoxic potential.

Table 5.2-1: Summary of acute toxicity, irritation and sensitisation studies (new studies not yet submitted highlighted in black and bold – studies in the baseline dossier in gray)

Study Reference	Species	Vehicle	Sex	LD/LC$_{50}$
Acute oral toxicity				
[redacted] Sept 1978 M-094154-01-1	Rat	Peanut oil	Males	50 mg/kg
			Females	31 mg/kg
[redacted] May 1989, M-149276-01-1	Rat	1% methylcellulose	Males	>5000 mg/kg
			Females	>5000 mg/kg
[redacted] Aug. 1996, M-139700-01-1	Rat	Corn oil	Males	95 mg/kg
			Females	87 mg/kg
[redacted]., Dec 2005, M-263224-01-1	Rat	0.5% carboxymethyl cellulose	Females	>2000 mg/kg
Acute dermal toxicity				
[redacted] Feb. 1979, M-101629-01-1	Rat	1% aqueous methylcellulose	Males	>2940 mg/kg
			Females	>2940 mg/kg
[redacted] Sept. 2000, M-199039-02-1	Rat	-	Males	>2000 mg/kg
			Females	>2000 mg/kg
[redacted] Oct 2005, M-258954-01-1	Rat	-	Males	>2000 mg/kg
			Females	>2000 mg/kg
Acute inhalation toxicity				
[redacted], June 1978, M-101619-01-1	Rat	-	Males	0.6 g/m^3 6 hours
			Females	0.6 g/m^3 6 hours
[redacted], July 1990, M-149264-01-1	Rat	-	Males	2.2 mg/l
			Females	2.2 mg/l
Skin irritation				
[redacted] Nov 1979, M-227752-01-1	Rabbit	-	Males	Non irritant
[redacted] Apr 1989, M-175955-01-1	Rabbit	-	Males	Non irritant
			Females	Non irritant
[redacted], Oct 2005, M-260123-01-1	Rabbit	-	Females	Non irritant

Study Reference	Species	Vehicle	Sex	LD/LC$_{50}$
Eye irritation				
[redacted], Nov 1976, M-227753-01-1	Rabbit	-	Males	Non irritant
[redacted] Apr 1989, M-149290-01-1	Rabbit	-	Males	Non irritant
			Females	Non irritant
[redacted], **Nov 2005, M-260858-01-1**	Rabbit		Females	Non irritant
Skin sensitisation				
[redacted] Sep 1977, M-227645-01-1	Guinea pig (M&K)	-	Males	Not sensitiser
			Females	Not sensitiser
[redacted] Sep 1989, M-175951-01-1	Guinea pig (Buehler)	1% aqueous methylcellulose	Males	Not sensitiser
			Females	Not sensitiser
[redacted], **Nov 2005, M-261562-01-1**	Guinea pig (Buehler)	PEG400	Females	Not sensitiser
Acute intravenous toxicity				
[redacted], June 1992, M-138700-01-1	Rat	PEG300	Females	Mean lethal dose = 23.9 mg/kg
[redacted], June 1992, M-138697-01-1	Laying hens	PEG 300	Females	Mean lethal dose = 4.4 mg/kg
Acute intraperitoneal toxicity				
[redacted] 1978 M-094154-01-1	Rat	Peanut oil	Males	50 mg/kg

***In vitro* Phototoxicity**

Study Reference	Cell Line	Results
In vitro Phototoxicity [redacted], 2013 M-466174-01-1	Balb/c 3T3	Negative

Comparison with the classification criteria

The results of the acute oral toxicity studies in rats show the influence of the vehicle on the LD50. In oils the LD50 are below 100 mg/kg whereas in aqueous vehicles, they are above, at least, 2000 mg/kg. Considering all the studies and due to deficiencies observed in some of them, deltamethrin is classified under current harmonized EU classification, according to the CLP regulation EC 1272/2008, Acute Tox Cat3, H301 (toxic if swallowed) based on the LD50 of 87 mg/kg, from the [redacted]'s study.

Based on the different acute dermal toxicity studies, deltamethrin is not acutely toxic through the dermal route and doesn't warrant any classification.

Several acute inhalation toxicity studies were performed with deltamethrin with a LC50 range from 0.6 to 2.2 mg/L. According to the CLP regulation EC 1272/2008, deltamethrin is classified Acute Tox Cat 3, H331 (toxic if inhaled) based on the LC50 of 0.6 mg/L from the [redacted]'s study.

Based on the different skin irritation studies in rabbits, deltamethrin is not irritant to the skin and doesn't warrant any classification.

Based on the different eye irritation studies in rabbits, deltamethrin is not irritant to the eyes and doesn't warrant any classification.

Based on the different skin sensitisation studies in guinea pigs, deltamethrin is not a skin sensitiser and doesn't warrant any classification.

Based on the cytotoxicity assay in vitro on BALB/c 3T3 cells, deltamethrin does not possess any phototoxic potential.

Conclusions on classification and labelling

CLP Regulation: Acute Tox 3, H301 (Toxic if swallowed)
Acute Tox 3, H331 (Toxic if inhaled)

CA 5.2.1 Oral

In addition to the acute oral toxicity studies already available in the Monograph and baseline dossier a new acute oral toxicity study was conducted in 2005 in order to support a registration in Brazil. This new study is summarized below. For the already submitted studies, a copy of the summaries performed by the former RMS Sweden available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report: KCA 5.2.1/01; [redacted]; 1979; M-094154-01-1
Title: Toxicity studies with decamethrin, a synthetic pyrethroid insecticide.
Report No.: A20968
Document No.: M-094154-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: no
***Annex data point due to first Annex I listing** *Experimental design*

Oral toxicity

Deltamethrin (purity not specified) was dissolved in peanut oil and administered via gavage to adult (3-4 months of age) male and female and weanling female (4-6 weeks of age) Sherman rats. The lowest dose tested was 30 mg/kg bw for adult male rats, 5 mg/kg bw for adult female rats and 7.5 mg/kg bw for weanling female rats. Comment: Other dose levels used were not given in the article. The observation period was 14 days.

Dermal toxicity

Deltamethrin (purity not specified) was dissolved in xylene and administered to the intact but shaved skin of adult female Sherman rats. After 5 days of restraint in a Bollman type cage (to prevent treating and licking of the treated area) the rats were removed, and washed with detergent and warm water. The dose administered was 800 mg/kg bw. Comment: Other dose levels used were not given in the article. The observation period was 14 days.

Inhalation toxicity

Male and female Sprague-Dawley rats were exposed whole body in exposure chambers to deltamethrin (purity not specified) aerosols generated from 10% DMSO solutions. Ten animals of each sex for each dose level were used. Comment: The dose levels used were not given in the article. The observation period was 14 days.

Intravenous toxicity

Deltamethrin (purity not specified) was dissolved in acetone and administered via a single injection into the tail vein of adult female and weanling female Sherman rats. The lowest dose administered was 1.6 and 0.8 mg/kg bw, for adult females and weanling females respectively. Comment: *Other dose levels used were not given in the article.* The observation period was 14 days.

Results

Oral toxicity

LD_{50} was estimated at 52 mg/kg bw for adult male rats, 31 mg/kg bw for adult females and 50 mg/kg bw for weanling females (95% confidence limits: 46-58, 29-34 and 42-60 mg/kg bw, respectively). The minimum toxic doses were 30 mg/kg bw for males (moderate to severe salivation and convulsions), 10 mg/kg bw for adult females (mild salivation) and 15 mg/kg bw for weanling females (mild salivation). Comment: *There are no indications whether lower doses than 30 mg/kg bw were tested on male rats.* Clinical signs of toxicity included salivation (often accompanied by irregular breathing), ataxia and convulsions. These primary signs of toxicity did not persist into the second day for most animals. Weakness, dyspnea, anorexia and staining of the fur were observed beyond the first day. In a few animals, the primary signs occurred 24-28 hours after administration. Recovery was most rapid in weanling females (most of the animals were normal after 20 hours and all within 44 hours) and slowest in adult males (some residual effects were evident up to 5 days after administration). Gross examination of the animals that died showed congestion of the lungs and adrenals. In some animals flatulence of the stomach and intestines was observed. No grossopathological changes were observed in animals necropsied 14 days after exposure to deltamethrin.

Dermal toxicity

LD_{50} was estimated at >800 mg/kg bw since this dose did not produce any signs of toxicity.

Inhalation toxicity

LC_{50} for female rats was estimated at 785 mg/l air and for male rats at 940 mg/l air. According to the author, clinical signs of toxicity and results from the gross examination were similar to those obtained by oral exposure.

Intravenous toxicity

LD_{50} for weanling female rats was estimated at 1.8 mg/kg bw and for adult female rats at 3 mg/kg bw (95% confidence limits: 1.5-2.1 and 2.9-5.3 mg/kg bw, respectively). According to the author, clinical signs of toxicity and results from the gross examination were similar to those obtained by oral exposure. The rate of onset was most rapid with intravenous administration.

Comments from the former RMS Sweden

The reference is a published article (J. Environ. Pathol. Toxicol. 2: 751-765, 1979) which consists of a summary of toxicity studies on deltamethrin conducted by the E.P.A. United States. No raw data were available. There are several serious shortcomings concerning the acute toxicity studies in this reference when compared with the OECD guidelines. No dose levels or just the lowest dose levels used were given. The number of animals used was not specified except for the inhalation toxicity study. The given dose high level in the dermal toxicity study was too low. The exposure time was not given in the inhalation toxicity study, and there are lack of data concerning the inhalation equipment and physical measurements. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the studies were performed). The studies were not of acceptable quality due to lack of important data. Although there were some lack of data in the article concerning the specification of dose levels or the number of animals used in the acute oral toxicity study, the LD_{50} values seem to be comparable with LD_{50} values of a similarly oral toxicity studies reported herein using oils as vehicle. The results of the acute oral toxicity study were therefore taken into consideration in the report. The result of the acute dermal toxicity study was also taken into consideration in this report due to the fact that the result was comparable with similar results reported in the literature. LD_{50} of 700 mg/kg bw was cited in IPCS International Programme on Chemical Safety, WHO, 1990. Deltamethrin. Environmental Health Criteria 97. *Comment: No details concerning this study were available).*

Report: KCA 5.2.1/01; [redacted]; 1989; M-149276-01-1
Title: Acute oral toxicity study of deltamethrin in rats
Report No.: A70785
Document No.: M-149276-01-1
Guideline(s): USEPA (=EPA): 81-1
Guideline deviation(s): not specified
GLP/GEP: **yes**

Experimental design

Five male and five female rats (Sprague-Dawley, [redacted]) were administered deltamethrin (purity 99.2%) once by oral gavage, as a wt/vol suspension in 1% methylcellulose at a dose level of 5000 mg/kg bw. The dose volume was 10 mg/kg. The observation period was 14 days. Necropsy was performed on all animals sacrificed at termination.

This study followed OECD 401 which is not in place anymore. The test article-related data are in agreement with the current OECD guidelines 420 and 423 which cover acute toxicity testing. Also animal-related data are sufficient, 5 animals per group of both sexes were used. The compound was suspended with a vehicle which is an accepted procedure by OECD 420 and 423 for accurate dosing in standardized application volumes. The LD_{50} value is in line with other acute studies where deltametrhin was administered in aqueous vehicle, also with a recently conducted acute toxicity study from [redacted]; 2005; M-263224-01-1.

Results

LD50 for male and female rats was calculated to be greater than 5000 mg/kg bw. There was no mortality in this study. There were no clinical signs of toxicity and no remarkable changes in body weights. No gross pathological changes were observed at necropsy.

Comments from the former RMS

The results of this study point out the importance of the choice of vehicle. Deltamethrin seems to be poorly absorbed in the rat when an aqueous suspension in methylcellulose was used as the vehicle. The study follows OECD guideline no 401 except for the fact that a suspension of deltamethrin was used and not the undiluted test substance. This fact severely restricts the sensitivity of the test. The study was conducted in accordance with the principles of GLP and submitted to Quality Assurance inspections. The results give some information about the great influence of the vehicle on the LD50, and was therefore taken into consideration in this report. However, the study is not of acceptable quality for a classification of the acute oral toxicity of deltamethrin due to the choice of suspended test substance instead of undiluted deltamethrin.

Report: KCA 5.2.1/04; [redacted]; 1996; M-139700-01-1
Title: Acute oral toxicity study of Deltamethrin in albino rats
Report No.: A55812
Document No.: M-139700-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **yes**

Experimental design

Deltamethrin (purity 98%) was dissolved in corn oil and administered orally by gavage as a single dose to nonfasted rats at levels of 50, 75, 100 and 150 mg/kg bw. Each group consisted of five male and five female rats (Sprague-Dawley Crl: CD BR).

Also in this study the test article-related data are in agreement with the current OECD guidelines 420 and 423. Five animals per group of both sexes were used, they were not fasted as required by the guidelines. Also in this study the use of a vehicle is a normal procedure for accurate dosing in standardized application volumes and also accepted by OECD 420 and 423. The LD_{50} value is in line with other acute studies.

Results

LD_{50} was estimated at 95 mg/kg bw for nonfasted male rats, and 87 mg/kg bw for nonfasted female rats (95% confidence limits were 74-122 mg/kg bw and 77-97 mg/kg bw for males and females, respectively). Mortality in the 50, 75, 100 and 150 mg/kg bw groups was 0/5, 0/5, 5/5 and 4/5, respectively for the males, and 0/5, 0/5, 5/5 and 5/5, respectively, for the females. All deaths occurred by day 1. The clinical observations in the 50 and 75 mg/kg bw groups and the surviving 150 mg/kg bw group male on day 0 included gait alterations, impaired righting reflex, reduced or absent forelimb/hindlimb grasp, repetitive convulsions, writhing and walking with splayed hindlimbs, repetitive jaw movement, vocalization, animal appeared flattened with limbs extended, salivation, lacrimation and chromodacryorrhea. The only behavioral alteration persisting to day 1 were reduced forelimb and hindlimb grasp for one male in the 75 mg/kg bw group. Other treatment-related clinical signs observed at the post-dosing time points for the surviving animals consisted of red, clear and/or yellow matting/material on various body surfaces. In general these signs did not persist beyond day 2.

Scrabbing on the neck was observed for two females in the 50 mg/kg bw group. No other gross external or internal lesions were noted.

Comments from the former RMS Sweden

The study follows OECD guideline no 401 except for the fact that the animals were not fasted prior to substance administration. However, the LD_{50} values estimated in this study seem to be comparable with LD_{50} values of similar acute oral toxicity studies reported herein, using oils as vehicle. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.2.1/05; [redacted], 2005; M-263224-01-1
Title:	Deltamethrin technical - Acute toxicity in the rat after oral administration
Report No.:	AT02671
Document No.:	M-263224-01-1
Guideline(s):	OECD 423 (2001) EEC 67/548 Annex V- Method B.1 tris EPA OPPTS 870.1100
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: Deltamethrin technical

Article no.:	AE F032640 00 1D99 0025
Description:	light-yellow powder
Lot/Batch no:	EDDLTO038
Purity:	99.9%
Stability of test compound:	guaranteed for study duration, expiry date: 2007-07-11

2. Vehicle: 0.5% aqueous carboxymethylcellulose-sodium

3. Test animals:

Species:	Rat
Strain:	HsdCpb:Wu (Wistar)
Age:	10-12 weeks approximately
Weight at dosing:	164 g – 182 g
Source:	[redacted]
Acclimatisation period:	at least 5 days
Diet:	[redacted] Maus/Ratte Haltung, [redacted] Switzerland, ad libitum
Water:	tap water, ad libitum
Housing:	group caged conventionally in polycarbonate cages on low dust wood granulate bedding ([redacted], Germany)
Environmental conditions:	Temperature: 22 ± 2 °C
	Humidity: 55 ± 5%
	Air changes: Approximately 10 changes per hour
	Photoperiod: Alternating 12-hour light and dark cycles

B. Study Design and methods

1. In life dates

14 September to 05 October 2005

2. Animal assignment and treatment

The substance was tested using a stepwise procedure, each step using three female rats. The animals were assigned to their groups by randomization. The random list was based on evenly distributed chance numbers by a software application. Following an overnight fast (16 to 24 hours), one group received a single dose of 2 000 mg/kg bw of deltamethrin by gavage. As no mortality was observed a second group was administered at the dose level. The test substance was administered in 0.5% aqueous carboxylmethylcellulose-sodium at a volume of 10 mL/kg bw. Clinical signs and mortality rates were determined several times on the day of administration and subsequently at least once daily for an observation period of at least 14 days. Body weights were recorded on days 1, 8 and 15. On day 15, surviving animals were sacrificed and all animals were necropsied and examined for gross pathological changes.

Table 5.2.1-01: Doses, mortality /clinical signs/ animals treated

Dose (mg/kg bw)	Toxicological results*	Occurence of signs	Mortality (%)
2 000 (1st)	0/0/3	-	0
2 000 (2nd)	0/0/3	-	0

* : number of animals which died spontaneously and/or were sacrificed in moribund state/number of animals with signs of toxicity/total number of animals used per group

3. Statistics

The data did not warrant statistical analysis.

II. Results and discussion

A. Mortality

Details are provided in Table 5.2.1-01. The dose of 2 000 mg/kg bw induced no mortality.
The oral LD_{50} cut-off was 5 000 mg /kg bw according to OECD guideline 423.

B. Clinical observations

No clinical signs were observed.

C. Body weight

There was no toxicological effect on body weight or body weight.

D. Necropsy

No abnormalities were observed at gross necropsy.

III. Conclusions

The oral LD_{50} cut off of deltamethrin administered in 0.5% aqueous carboxymethylcellulose-sodium was 5000 mg/kg bw (GHS Category 5).

CA 5.2.2 Dermal

In addition to the acute dermal toxicity studies already available in the Monograph and baseline dossier a new acute dermal toxicity study was conducted in 2005 in order to support a registration in Brazil. This new study is summarized below. For the studies already submitted, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.2.2/01; [redacted]; 1979; M-101629-01-1
Title:	Acute percutaneous toxicity to rats of decamethrin.
Report No.:	A28974
Document No.:	M-101629-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Five male and five female rats (Sprague Dawley, CD strain) were topically administered deltamethrin (purity not specified) on intact skin at 2940 mg/kg bw. The test article was prepared as a 60% wt/vol suspension in aqueous methylcellulose (1%). The control animals (five female and five male rats) received the vehicle, only.

In this study all technical parameters are in agreement with the current OECD guidelines 402. GLP was not obligatory at that time, but the study director stated that the work was performed under his supervision according to the procedures, and that this report provides a correct and faithful record of the results obtained. Five animals per group of both sexes were used according to the guideline. Also in this study the use of a vehicle is a normal procedure for accurate dosing and also accepted by OECD 402. The LD_{50} value is in line with other acute studies, also with a recent study ([redacted]; 2005; M-258954-01-1).

Results

LD_{50} was estimated as >2940 mg/kg bw. There were no mortalities or signs of reaction to the treatment. There were no observable dermal reactions at the site of application in either treated or control rats. Body weight gains for four treated females were depressed during the first week of observation, compared with the controls, but returned to normal during the second week of observation. Terminal autopsy findings were within normal limits.

Comments from the former RMS

The result indicates that an aqueous suspension of deltamethrin in 1% methylcellulose was poorly absorbed through the skin, since the acute toxicity was low in this study. The study follows OECD guideline no 402 with exception of lack of raw data, and lack of data concerning housing- and feeding conditions. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). A serious shortcoming concerning this study is that deltamethrin was investigated for acute dermal toxicity using a *suspension* of deltamethrin and not the undiluted *test* substance, which is preferable. This fact severely restricts the sensitivity of the test. The study is not of acceptable quality for classification of the acute dermal toxicity of deltamethrin due to the choice of suspended test substance instead of undiluted deltamethrin.

Report:	KCA 5.2.2/02; [redacted]; 2000; M-199039-02-1
Title:	Acute dermal toxicity in rats deltamethrin
Report No.:	C009679
Document No.:	M-199039-02-1
Guideline(s):	EU (=EEC): 92/69 B.3; OECD: 402
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Deltamethrin (98.6%) was dissolved in corn oil and topically administered to the intact but shaved skin of five male and five female rats (Wistar RCC:WI (IOPS HAN/HAN)). The dose administered was 2000 mg/kg bw. The observation period was 14 days. All animals were subjected to gross pathological examination.

This study was compliant with the current OECD guidelines 402. The LD_{50} value is in line with other acute studies, also with a recent study ([redacted]; 2005; M-258954-01-1).

Results

LD_{50} was calculated to be greater than 2000 mg/kg bw. There were no mortalities. No clinical signs and no cutaneous reactions were observed during the study. Macroscopic examination revealed no apparent abnormalities in the animals.

Comments from the former RMS

The study follows OECD guideline no. 402. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.2.2/05; [redacted]; 2005; M-258954-01-1
Title:	Deltamethrin technical - Acute toxicity in the rat after dermal application
Report No.:	AT02461
Document No.:	M-258954-01-1
Guideline(s):	OECD 402 (1987) EEC 67/548 Annex V- Method B.3; EPA OPPTS 870.1200, EPA 712-C-98-192 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: Deltamethrin technical

Article no.:	AE F032640 00 1D99 0025
Description:	light-yellow powder
Lot/Batch no:	EDDLTO038
Purity:	99.9%
Stability of test compound:	guaranteed for study duration; expiry date: 2007-07-11

2. Vehicle: Not applicable

3. Test animals:

Species:	Rat
Strain:	Crl:(Wi) Wu BR (Wistar)
Age:	9 -13 weeks approximately
Weight at dosing:	231 to 262 g for males, 197 to 209 g for females
Source:	[redacted], [redacted]
Acclimatisation period:	at least 5 days
Diet:	[redacted] Maus/Ratte Haltung, [redacted] Switzerland, ad libitum
Water:	tap water, ad libitum
Housing:	caged individually in polycarbonate cages on low dust wood granulate bedding ([redacted], [redacted], Germany)
Environmental conditions:	Temperature: 22 ± 2°C
	Humidity: 55 ± 5%
	Air changes: Approximately 10 changes per hour
	Photoperiod: Alternating 12-hour light and dark cycles

B. Study Design and methods

1. In life dates: 17 to 31 August 2005

2. Animal assignment and treatment

Animals were assigned by randomization to the test groups listed in Table 5.2.2-01. The random list was based on evenly distributed chance numbers especially generated for the study by a software application. On the day prior to dosing, the fur was clipped from the dorsal area of the trunk of each animal (approximately 10% of the body surface area). The test substance was administered as a single occluded dermal application and was applied moistened with distilled water. After an exposure period of 24 hours, the occlusion was removed and residual test material was removed with tepid water using soap and gently patting the area dry. Animals were observed for clinical signs and mortality several times on the day of dosing and subsequently at least once daily for an observation period of at least 14 days. Individual body weights were recorded on days 1, 8 and 15. On day 15, all animals were sacrificed by carbon dioxide and were necropsied and examined for gross pathological changes.

Table 5.2.2-01: Doses, toxicological results* / animals treated

Dose (mg/kg bw)	Male	Female	Combined
2000	0/0/5	0/0/5	0/0/10

*** : number of animals which died spontaneously and/or were sacrificed in moribund state/number of animals with signs of toxicity/total number of animals used per group**

3. Statistics

The data did not warrant statistical analysis.

II. Results and discussion

A. Mortality

Details are provided in Table 5.2.2-01. No mortalities occurred at 2000 mg/kg bw, the only dose level tested.

The dermal LD_{50} for males was > 2000 mg/kg bw
for females was > 2000 mg/kg bw
for the combined sexes was > 2000 mg/kg bw.

B. Clinical observations

No clinical signs were observed during the study.

C. Body weight

Body weight and body weight gain of male or female rats were not affected by treatment.

D. Necropsy

The necropsies performed at the end of the study revealed no particular findings.

III. Conclusions

The dermal LD_{50} of deltamethrin was higher than 2000 mg/kg bw in both sexes (GHS category 5; unclassified).

CA 5.2.3 Inhalation

All necessary acute toxicity studies were presented and evaluated during the EU process for Annex I listing. However, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.2.3/01; [redacted]; 1990; M-149264-01-1
Title:	Acute inhalation toxicity evaluation of deltamethrin in rats
Report No.:	A70770
Document No.:	M-149264-01-1
Guideline(s):	USEPA (=EPA): Subdivision F, 81-3
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Experimental design

Groups of five male and five female Sprague Dawley derived albino rats ([redacted]), were exposed whole body for a single four hour period to dust paniculate aerosol atmospheres of deltamethrin (purity not specified) at concentrations of 1.0, 1.8 and 2.3 mg/1. An aerosol of the test material was characterized by an mass median aerodynamic diameter of 3.7 \xm with a geometric standard deviation of 1.87.

This study fulfils almost all requirements (except purity of compound) of OECD guideline 403 (7 Sept 2009) and was conducted under GLP conditions. The exposure duration of 4 hours, the doses, the particle examinations and the group sizes are in agreement with the guideline. The lethality data are given for both sexes, however, from the individual tables the lethality data can be spilt between the sexes. For males the lethality was was 2/5, 2/5 and 0/5 at 1.0, 1.8 and 2.3 mg/L air, respectively and for females 1/5, 2/5 and 1/5 at 1.0, 1.8 and 2.3 mg/L air, respectively. Therefore, no major sex differences were obvious so that the combined determination of a LC_{50} is justified and are a good basis for setting of an LC_{50}.

Results

LC50 for male and female rats combined was estimated at 2.2 mg/1 (95% confidence limits: 1.5-3.3 mg/1). The most significant clinical findings were impaired hind limb function, labored breathing, increased salivation and hunched posture. Animals in all groups lost body weight during the first post-exposure week. Enlarged inguinal and mandibular lymph nodes and pulmonary congestion were observed.

Comments from the former RMS Sweden

The study follows OECD guideline no 403 except for the fact that the LC30 for rats was determined for males and females combined and not for each sex. The purity of the test substance was not

specified. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.2.3/02; [redacted]; 1978; M-101619-01-1
Title:	RU 22974 - Acute inhalation toxicity in rats. 6 hour LC 50.
Report No.:	A28960
Document No.:	M-101619-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Groups of seven male and seven female albino rats (Sprague Dawley, CD strain) were exposed whole body for a single six hour period to dust particulate aerosol atmospheres of deltamethrin (purity not specified) at concentrations of 0 (air only), 0.049, 0.430, 0.540 and 0.720 mg/l air. About 69-86% of the total aerosol had a mean aerodynamic diameter of less than 2.5 µm. The observation period was 14 days.

This study fulfils most of the requirements of OECD guideline 403 (7 Sept 2009). It was non-GLP, but in the spirit of GLP based on a respective declaration of the study director that the work was performed under his supervision according to the procedures described, and that his report provides a correct and faithful record of the results obtained. The purity of the compound was not given. The exposure duration of 6 hours, the doses, the particle examinations and the group sizes are in agreement with the guideline. The lethality data are summarized for both sexes together, from the individual tables the lethality for males was 3/7 and 6/7 at 0.540 and 0.720 mg/L air, respectively and for females 2/7 and 6/7 at 0.540 and 0.720 mg/L air, respectively. Therefore, no sex differences were obvious so that the combined determination of a LC_{50} is justified. The data are a solid basis for establishment of an LC_{50}.

Results

LC_{50} (6 hour) for male and female rats combined was estimated at 0.6 mg/l. Clinical signs included skin and eye irritation, agitated grooming, ptyalism, diaphragmatic breathing, stained fur, ataxia and hypersensitivity. Animals in all treated groups lost body weight after exposure. By day 5 of the 14 day observation period bodyweight gain was normal for all animals. Food consumption was decreased for animals in all treated groups for up to six days after exposure. Gas filled stomachs, massive haemorrage and oedema of the lungs, mucus and blood within the lumen of the trachea and the test substance present as a white deposit in the larynx and trachea were noted at the macroscopic examination.

Comments from the former RMS Sweden

There are some deviations from OECD guideline no 403. The purity of the test substance was not specified. The LC_{50} for rats was determined for males and females combined and not for each sex. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed). The complete report was not available at the time of evaluation (no raw data was available). The study seems to be of acceptable quality. The Commission

working group on the classification and labelling of dangerous substances-pesticides has proposed a classification of deltamethrin with R 23 based on the study report.

CA 5.2.4 Skin irritation

In addition to the skin irritation studies already available in the Monograph and baseline dossier a new skin irritation study was conducted in 2005 in order to support a registration in Brazil. A detailed summary of this new study is provided in this chapter. For the studies already submitted, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.2.4/01; [redacted]; 1979; M-227752-01-1
Title:	RU22974 - Test to determine primary cutaneous irritation in the rabbit
Report No.:	A95068
Document No.:	M-227752-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

A test for dermal irritation of RU 22974 (deltamethrin) (purity 98%) was carried out using 0.5 g of the test substance applied to the intact but shaved skin on the left flank of 12 male albino New Zealand White rabbits. Additionally, 0.5 g was applied on the right flank of each rabbit where the epidermis had been scarified. The exposure time was 23 hours. One hour later, the primary irritation index was evaluated. Forty-eight hours later a second reading was made. The irritation index was evaluated according to the system of Draize. According to the author, a classification scale recommended by the "Journal Officiel de la Republique Francaise" of 21/4/71 and 5/6/73 was followed.

This study covers most requirements of OECD 404, the examinations were only performed after 1 and 48 hour, but this covered the time of a possible reaction and none occurred. Importantly, the result is in agreement with results of a recently conducted skin irritation study ([redacted]; 2005; M-260123-01-1).

Results

No erythema or oedema was observed on any of the animals at 1 hour or 48 hours after finished exposure. The primary irritation index was 0.0 indicating the test article to be non-irritating to the skin of rabbits.

Comments from the former RMS

Based on this study, deltamethrin was not classified as an irritant to rabbit skin. The study follows OECD guideline no 404 except for some minor deviations. The observation period was 1 h and 48 h (according to OECD guideline the animals should be examined for irritative response at 1 h, and then at 24, 48 and 72 h). The temperature and humidity in the animal room were not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory when this study was performed). However, the study seems to be of acceptable quality.

Report:	KCA 5.2.4/02; [redacted]; 1989; M-175955-01-1
Title:	Primary dermal irritation test of deltamethrin in rabbits
Report No.:	A98131
Document No.:	M-175955-01-1
Guideline(s):	USEPA (=EPA): 81-5
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

A test for dermal irritation of deltamethrin (purity 99.2%) was carried out using 0.5 g of the test substance "slightly moistened" with 1% aqueous methylcellulose applied on the intact skin on the back of each 6 albino New Zealand White rabbits, 2 animals/sex for 4 hr exposure. The Draize scale was used to assess the degree of erythema and oedema.

This study followed OECD 404, the examinations were performed in agreement with the guideline requirements. It is stated that the animal rooms are with controlled temperature, humidity and light (hours light and 12 hours dark), diet and water were freely available.
Importantly, the result is in agreement with results of a recently conducted skin irritation study ([redacted], 2005).

Results

There were no erythema or oedema observed in any of the animals at 30 minutes or at 24,48 and 72 hours after exposure. The primary irritation index was 0.0, indicating the test article to be non-irritating to the skin of rabbits.

Comments from the former RMS

Based on this study, deltamethrin was not irritating to the skin of rabbits. The study follows OECD guideline no 404, except for lack of data concerning the housing conditions (temperature and humidity in the animal room were not specified). The study was conducted in accordance with the principles of GLP and submitted to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.2.4/03; [redacted]; 2005; M-260123-01-1
Title:	Deltamethrin technical - Acute skin irritation/corrosion on rabbits
Report No.:	AT02547
Document No:	M-260123-01-1
Guideline(s):	OECD 404 (2002); EEC 67/548 Annex V- Method B.4 (1967); EPA OPPTS 870.2500, EPA 712-C-98-196 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

A. Materials

1. Test material:	Deltamethrin technical
Article no.:	AE F032640 00 1D99 0025
Description:	White powder
Lot/Batch no:	EDDLTO038
Purity:	99.9%
Stability of test compound:	guaranteed for study duration, expiry date: 2007-07-11
2. Vehicle:	Not applicable
3. Test animals:	
Species:	Rabbit, females only
Strain:	Esd:NZW
Age:	Young adult animal
Weight at dosing:	2.1 to 2.5 kg
Source:	[redacted] France, [redacted]
Acclimatisation period:	at least 5 days
Diet:	standard diet "Ssniff K-Z" 4mm ([redacted] Germany), approximately 100 g per animal per day
Water:	tap water, ad libitum
Housing:	housed individually in cage units Metall/Noryl by EBECO
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 50 ± 25% Photoperiod: Alternating 12-hour light and dark cycles

B. Study Design and methods

1. In life dates: 11 to 14 October 2005.

2. Animal assignment and treatment

This testing strategy comprised a stepwise approach including the evaluation of existing human and/or animal data showing effects on the skin or mucous membranes, the performance of a SAR evaluation for skin corrosion/irritation, measurement of pH value, the evaluation of data on systemic toxicity via the dermal route and the performance of a validated in vitro test for skin corrosion (Human 3D Epidermal Skin Model) before in vivo testing for skin irritation/corrosion in rabbits. The test compound is not corrosive to the skin.

On the day before the test, the fur was shorn on the right and left side from the dorso-lateral area of the trunk of each of the rabbits. Care was taken to avoid abrading the skin. Only animals with healthy and intact skin were used.

0.5 g of the pulverized test substance moistened with Aqua p.i. (to ensure good contact with the skin) was applied to the skin of the animal under a gauze patch. The treated skin area was approximately 2.5

cm by 2.5 cm in size. The patch was placed on the dorso-lateral areas of the trunk of each animal and was held in place with non-irritating tape for the duration of the exposure period. After the exposure period the dressing and patch were removed. The exposed skin area was carefully washed with water. The contralateral skin area not treated with test substance served as control.
Due to a possible irritant potential of the test substance, in the first step only one animal was used and three test patches were applied successively to this animal, as described above. The first patch was removed after three minutes. As no serious skin reactions were observed, the second patch was removed after one hour. At this stage the observations indicated that with respect to animal welfare the exposure can be allowed to extend to four hours, therefore the third patch was removed after four hours and the responses were graded one hour later. The test was completed using two additional animals, exposed for four hours.
The dermal irritation was scored approximately at 1, 24, 48 and 72 hours after patch removal. As no irritation indices were observed after 72 h, the study was finished.
The degree of erythema/eschar formation and oedema formation was recorded as specified by DRAIZE and any serious lesions or toxic effects other than dermal irritation were also recorded and fully described. As general criteria the body weight of each animal was recorded at the beginning of the study. If clinical findings other than dermal irritations occur they were recorded daily.

II. Results and discussion

A. Findings

No erythema, eschar or oedema was observed at any time point.

Table 5.2.4-01: Individual skin irritation scores according to the Draize scheme on the first animal

Observation (immediately after patch removal)	Duration of exposure	
	3 minutes	1 hour
Erythema (redness) and eschar formation	0	0
Oedema formation	0	0

Table 5.2.4-02: Individual and mean skin irritation scores after 4 hour exposure according the Draize scheme

	Erythema and eschar			Oedema		
Animal number (body weight in kg)	**1 (2.1)**	**2 (2.5)**	**3 (2.5)**	**1 (2.1)**	**2 (2.5)**	**3 (2.5)**
1 hour	0	0	0	0	0	0
24 hours	0	0	0	0	0	0
48 hours	0	0	0	0	0	0
72 hours	0	0	0	0	0	0
Mean score 24-72 hours	0			0		
No positive response: mean scores < 2 = - Positive response : mean scores ≥ 2 = +						

III. Conclusions

Deltamethrin technical was non-irritant to the rabbit skin and there were no systemic intolerance reactions. On the basis of this study, deltamethrin technical does not warrant classification as being irritating to the skin.

CA 5.2.5 Eye irritation

In addition to the eye irritation studies already available in the Monograph and baseline dossier a new eye irritation study was conducted in 2005 in order to support a registration in Brazil. A detailed summary of this new study is provided in this chapter. For the studies already submitted, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.2.5/01 [redacted]; 1976; M-227753-01-1
Title:	RU22974. Test to evaluate ocular irritation in the rabbit.
Report No.:	A95069
Document No.:	M-227753-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

A test for irritation of eyes of deltamethrin (purity 97%) was carried out by applying 0.1 g of the test substance into the conjunctival sac of the left eye of each twelve male New Zealand White albino rabbits. The eyes of six out of twelve rabbits were rinsed one minute after instillation of the test substance. The eyes of the other exposed rabbits (six males) were not rinsed. The irritation index was evaluated according to a scale similar to the system of Draize. The classification was made by using a scale established by IFREB (Institut Français de Recherches et Essais Biologiques).

This study mainly followed OECD 405, the examinations were performed in agreement with the guideline requirements. It is stated that the rabbits are kept either in individual cages measuring 600 x 540 x 315 mm, or in restraining devices and that the animal house is air-conditioned. Importantly, results of a recently conducted eye irritation study which did not show an eye-irriating effect are available ([redacted]; 2005; M-260858-01-1).

Results

In the rabbits whose eyes were not rinsed, swelling or obvious swelling of lids and nictating membranes, slight discharge and slight redness or redness of the conjunctivae were observed at 1 h, at 1 day and at 2 days after application. All animals had recovered on day four. In the animals whose eyes were rinsed, the same degree of irritation was observed at one hour after instillation but the animals had recovered already on day three. According to the scale established by IFREB, deltamethrin was classified as a slight irritant (maximum average score was 12.67 (at 1 h after instillation of the test substance) on a scale for scoring ocular lesions (cornea, iris, pupil and conjunctivae) where the total maximum score possible was 110).

Comments from the former RMS Sweden

Based on this study, deltamethrin was classified according to a scale established by IFREB as a slight irritant to the eyes of rabbits. However, the irritation of the eyes was not considered to be significant for a classification of deltamethrin as an eye irritant according to the directive 67/548/EEC. The study follows OECD guideline no 405 except for lack of data concerning the housing conditions (temperature and humidity in the animal room were not specified). There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). The study seems to be of acceptable quality.

Report:	KCA 5.2.5/02; [redacted]1989; M-149290-01-1
Title:	Eye irritation study of deltamethrin in rabbits.
Report No.:	A70799
Document No.:	M-149290-01-1
Guideline(s):	USEPA (=EPA): 81-4
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

A test for irritation of eyes of deltamethrin (purity 99.2%) was carried out by applying 87 mg of the test substance into the conjunctival sac of the right eye of each six New Zealand White rabbits (3 animals/sex). The Draize scale was used to assess the degree of irritation.

This study mainly followed OECD 405; the examinations were performed in agreement with the guideline requirements. Importantly, results of a recently conducted eye irritation study which did not show an eye-irriating effect are available ([redacted]2005; M-260858-01-1).

Results

Peak ocular irritation was observed at 1 h. The irritation generally consisted of moderate to marked conjunctival redness, slight to marked discharge and slight degrees of swelling. One animal additionally exhibited slight iridial irritation at 1 h. Clear discharge was observed during the study and was noted at 1 hr in three of the six rabbits. All animals had recovered after 72 h. According to the system of Draize, deltamethrin was classified as a slight irritant (maximum average score was 9.2 at 1 hr after instillation of the test substance on a scale for scoring ocular lesions (cornea, iris and conjunctivae) where the total maximum score possible was 110).

Comments from the former RMS Sweden

Based on this study, deltamethrin was classified as a slight irritant to the eyes of rabbits according to the system of Draize. However, the irritation of the eyes was not considered to be significant for a classification of deltamethrin as an eye irritant according to the directive 67/548/EEC. The study follows OECD guideline no 405. There were no indication whether the eyes were unwashed or not in the study. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.2.5/03; [redacted]; 2005; M-260858-01-1
Title:	Deltamethrin technical - Acute eye irritation on rabbits
Report No.:	AT02612
Document No.:	M-260858-01-1
Guideline(s):	OECD 405 (2002); EEC 67/548 Annex V - Method B.5 (1967); US EPA OPPTS 870.2400; EPA 712-C-98-195 (1998)
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: Deltamethrin technical

Article no.:	AE F032640 00 1D99 0025
Description:	white powder
Lot/Batch no:	EDELTO038
Purity:	99.9%
Stability of test compound:	guaranteed for study duration, expiry date: 2007-07-11

2. Vehicle: Not applicable

3. Test animals:

Species:	Rabbit, females only
Strain:	Hsd:NZW
Age:	Young adult animal
Weight at dosing:	2.5 to 2.7 kg
Source:	[redacted], France, [redacted]
Acclimatisation period:	at least 5 days
Diet:	standard diet "Ssniff K-Z" 4mm ([redacted], Germany), approximately 100 g per animal per day
Water:	tap water, ad libitum
Housing:	housed individually in cage units Metall/Noryl by EBECO
Environmental conditions:	Temperature: 20 ± 3 °C Humidity: 50 ± 25% Photoperiod: Alternating 12-hour light and dark cycles

B. Study Design and methods

1. In life dates

18 to 21 October 2005.

2. Animal assignment and treatment

The testing strategy comprised a stepwise approach including the evaluation of existing data, the performance of a SAR evaluation for eye and skin corrosion/irritation, measurement of pH value, the evaluation of data on systemic toxicity via the dermal route, the performance of a validated *in vitro* test for skin corrosion (Human 3D Epidermal Skin Model) and *in vivo* testing for skin irritation/corrosion in rabbits before *in vivo* testing for eye irritation/corrosion in rabbits. The test compound is not corrosive to the skin.

On the day before the test, both eyes of each animal were examined including fluorescein examination. Only animals with healthy intact eyes were used.
0.1 g of the pulverized test substance was placed into the conjunctival sac of one eye of the first animal after having gently pulled the lower lid away from the eyeball. The lids were gently held together for about one second in order to prevent loss of the test compound. The other eye, which remained untreated, served as control.
The eye was not rinsed for at least 24 hours following instillation.
If one hour after treatment a severe irritation was not observed, two further rabbits were treated as described.
Eye irritations were scored and recorded approximately at 1, 24, 48 and 72 hours post application. As no irritation indices were observed after 72 h, the study was finished. The degree of ocular lesions was recorded as specified by DRAIZE and any serious lesions or toxic effects other than ocular lesions were also recorded and fully described. As general criteria the body weight of each animal was recorded at the beginning of the study. If clinical findings other than eye irritations occur they were recorded daily.

II. Results

Redness of the conjunctivae was observed after 1 and 24 hours in all females (grade 2 for 3/3 females) and after 48 hours in one female (grade 2). Chemosis of the conjunctivae was occasionally observed in all females between 1 and 48 hours.

Table 5.2.5-01: Eye irritation scores according to the Draize scheme

Animal number (body weight in kg)	Cornea			Iris			Conjunctiva-redness			Conjunctiva-chemosis		
	1 (2.7)	2 (2.5)	3 (2.6)	1 (2.7)	2 (2.5)	3 (2.6)	1 (2.7)	2 (2.5)	3 (2.6)	1 (2.7)	2 (2.5)	3 (2.6)
Time of observation												
1 hour	0	0	0	0	0	0	2	2	2	0	1	1
24 hours	0	0	0	0	0	0	2	2	2	1	0	1
48 hours	0	0	0	0	0	0	2	0	0	1	0	0
72 hours	0	0	0	0	0	0	0	0	0	0	0	0
Mean scores 24-72 hours	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**1.3**	**0.7**	**0.7**	**0.7**	**0.0**	**0.3**

Conjunctivae: Redness (refers to palpebral and bulbar conjunctivae; excluding cornea and iris)
0: Normal
1: Some blood vessels hyperaemic (injected)
2: Diffuse, crimson color; individual vessels not easily discernible

Chemosis: Swelling (refers to lids and/or nictating membranes)
0: Normal
1: Some swelling above normal
2: Obvious swelling, with partial eversion of lids
3: Swelling, with lids about half closed
4: Swelling, with lids more than half closed

III. Conclusions

Slight ocular irritation was observed in all animals but had reversed by 72 hours. On the basis of this study, deltamethrin technical does not warrant classification as being an eye irritant in the EU.

CA 5.2.6 Skin sensitization

In addition to the skin sensitization studies already available in the Monograph and baseline dossier a new skin sensitisation study was conducted in 2005 in order to support a registration in Brazil. A detailed summary of this new study is provided in this chapter. For the studies already submitted, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report: KCA 5.2.6/01; [redacted]; 1977; M-227645-01-1
Title: RU22974 - decamethrine; Decis technical [redacted]. Sensitization test in the guinea pig.
Report No.: A28978
Document No.: M-227645-01-1
Guideline(s): --
Guideline deviation(s): --
GLP/GEP: **no**

Experimental design

The sensitizing potential of deltamethrin (RU 22974/Decis Technical) (purity not specified) was investigated in twenty albino (Hartley) guinea pigs (10 males and 10 females). The method used derived from the Guinea Pig Maximisation test. The induction comprised 10 closed-patch topical applications of the test substance (0.5 g undiluted deltamethrin, placed under occlusive patches for 48 h) and two intradermal injections of Freund's complete Adjuvant. The test substance was applied 3 times per week with a 2 day interval, for 3 weeks, and once at the start of the 4th week. The animals were challenged with the test article (0.5 g undiluted deltamethrin) two weeks after the induction phase by closed-patch topical application.

This study (non-GLP) covers the main requirements of OECD 406 and was from its design sufficiently sensitive to detect a sensitizing potential. It was negative and this is in line with results of a recently conducted sensitization study ([redacted]; 2005; M-261562-01-1).

Results

None of the animals responded following challenged with undiluted deltamethrin.

Comments from the former RMS Sweden

Based on this study, deltamethrin was not considered to be a sensitizing agent in the albino guinea pig. There are some deviations from the OECD guideline no 406. No positive/ negative control animals were used in the study. There was a lack of data concerning individual body weights and housing conditions. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). However the study seems to be of acceptable quality.

Report:	KCA 5.2.6/02; [redacted]; 1989; M-175951-01-1
Title:	Dermal sensitization study of deltamethrin in the Albino guinea pig (Buehler)
Report No.:	A98129
Document No.:	M-175951-01-1
Guideline(s):	USEPA (=EPA): 81-6
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

The sensitizing potential of deltamethrin (purity 99.2%) was investigated in twenty albino (Hartley) guinea pigs (10 animals/sex) according to the test method of Buehler. Additional animals were used as negative and positive controls (10 animals/sex/control group). The positive control animals received 2,4-dinitrochlorobenzene (0.01% concentration in acetone). The induction of sensitization was conducted by closed-patch topical applications of the test substance (0.4 g undiluted deltamethrin slightly moistened with 1% aqueous methylcellulose) for three consecutive weeks. The animals were challenged with the test article (0.4 g undiluted deltamethrin slightly moistened with 1% aqueous methylcellulose) two weeks later by closed-patch topical application. None of the animals responded following challenge with undiluted deltamethrin. The use of positive and negative controls gave expected results.

This GLP study (Buehler) covers the requirements of OECD 406 and was sufficiently sensitive to detect a sensitizing potential. It was negative and thus in agreement with results of a recently conducted sensitization study ([redacted]; 2005; M-261562-01-1).

Comments from the former RMS Sweden

Based on this study, deltamethrin was not considered to be a sensitizing agent in the albino guinea pig when 1% aqueous methylcellulose was used as vehicle. The study follows OECD guideline no 406 except for lack of data concerning the housing conditions (temperature and humidity were not specified). The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report: KCA 5.2.6/03; [redacted]; 2005; M-261562-01-1
Title: Deltamethrin technical (Project: Deltamethrin technical) - Study for the skin sensitization effect in guinea pigs (Buehler patch test)
Report No.: AT02618
Document No.: M-261562-01-1
Guideline(s): OECD N° 406 (1992); EEC 96/54/EC, Method B.6 (1996); EPA OPPTS 870.2600; EPA 712-C-03-197 (march 2003)
Guideline deviation(s): Analytical determinations of the stability of the paste in polyethylene glycol 400 for administration were not performed.
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test material: Deltamethrin technical

Article no.:	AE F032640 00 1D99 0025
Description:	white powder
Lot/Batch no:	EDDLTO038
Purity:	99.9%
Stability of test compound:	guaranteed for study duration; expiry date: 2007-07-11

2. Vehicle: PEG 400

3. Test animals:

Species:	Guinea pig, females only
Strain:	Crl:HA
Age:	5 - 6 weeks
Weight at dosing:	333 to 425 g
Source:	[redacted], Germany.
Acclimatisation period:	at least 5 days
Diet:	[redacted] Maintenance Diet for Guinea Pigs supplied by [redacted] ad libitum
Water:	tap water, ad libitum
Housing:	conventionally kept in type IV Makrolon® cages, in groups of five during the adaptation period and in groups of two or three per cage throughout the study period
Environmental conditions:	
Temperature:	22 ± 3 °C
Humidity:	55 ± 15%
Air changes:	At least 10 changes per hour
Photoperiod:	Alternating 12-hour light and dark cycles

B. Study Design and methods

1. Animal assignment and treatment:

Dose	
Topical induction:	83.3% (500 mg test item and 0.1 ml PEG 400)
Challenge:	83.3% (500 mg test item and 0.1 ml PEG 400)
Stability	stability and homogeneity of the test item formulations in the vehicle (1% - 50%) analytically verified for up to 2 hours.
Application route:	Dermal application on suitable areas of the body shaved 24 hours before each treatment
Application volume:	0.5 ml vehicle in the control group; 500 mg test item mixed with 0.1 ml vehicle in the test item group
Duration and schedule:	Three topical inductions with 7 days interval between each of them. After each dermal exposure of 6 hours, any remaining test item was removed with sterile physiological saline solution. The challenge was performed with the test item formulation 2 weeks after the last dermal induction by 6 hours dermal exposure on shorn flank of each animal in the control and test item group. Twenty one hours later the skin of the animals was shorn in the region of the treatment sites.
Group size:	32 females (control: 10, test item: 20, range-finding: 2)

Observations: mortality, clinical signs, skin reactions assessed 30 and 54 hours after the beginning of the challenge, body weight (at beginning and termination of study)

II. Results and discussion

Appearance and behaviour of the test item group were not different from the control group. One animal of the control group showed a labored breathing and a pale appearance at day 10. The animal died on day 11.
There were no skin effects in the animal of the test item group and the control group during the three induction treatments. By the end of the study the mean body weight of the treatment group animals was in the same range than that of the control group. The challenge with the 83.3% test item paste led to no skin effects in the animals of the test item group and no skin effects in the control group.

Table 5.2.6-1: Number of animals exhibiting skin effects

	Test item group (20 animals)					Control group (9 animals)				
	Test item patch			Control patch		Test item patch			Control patch	
Hours	**30**	**54**	**total**	**30**	**54**	**30**	**54**	**total**	**30**	**54**
Challenge 83.3%	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

III. Conclusion

Deltamethrin technical under the conditions of this test is not considered to be a dermal sensitizer and labeling for deltamethrin technical should not be required.

CA 5.2.7 Phototoxicity

Report:	KCA 5.2.7/01; [redacted]; 2013; M-466174-01-1
Title:	Deltamethrin TC: Cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight
Report No.:	1558000
Document No.:	M-466174-01-1
Guideline(s):	Commission Regulation (EC) No. 440/2008 B 41, dated May 30, 2008; Committee for Proprietary Medicinal Products (CPMP), Note for Guidance on Photosafety testing, EMEA, CPMP/SWP/398/01, adopted 27 June 2002, into operation in Dec 2002; OECD Guideline for Testing of Chemicals: Guideline: 432: In vitro 3T3 NRU phototoxicity test (Revised and approved by the National Co-ordinators in May 2002, approved by Council April 2004)
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

This study was performed to assess the phototoxic potential of deltamethrin TC. The test was performed using BALB/c 3T3 cells clone 31. In a first step a range finding experiment (RFE) was conducted, the second one was the main experiment (ME). The following concentrations of the test item were tested with and without irradiation in both experiments: 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100 µg/mL. As solvent control EBSS (Earle's Balanced Salt Solution) containing 1% (v/v) DMSO was used. Chlorpromazine was used as positive control. One test group of cells treated with the test item was irradiated with artificial sunlight for 50 minutes with 2.4 to 2.55 mW/cm2 UVA, resulting in an irradiation dose of 7.2 to 7.65 J/cm2 UVA. Another test group of test item treated cells were kept in the dark for 50 minutes.

Slight cytotoxic effects were observed after treatment of cells with the test item in the presence of irradiation with artificial sunlight in the RFE, but not in the absence of irradiation. Since the viability of the cells was not reduced below 50%, neither ED50-values nor a PIF could be calculated. The resulting MPE values were -0.006 (RFE). No cytotoxic effects occurred after treatment of the cells with the test item in the absence of irradiation with artificial sunlight. Therefore, ED50-values of a PIF could also not be calculated. The resulting MPE was -0.023 (ME) and thus, the test item is classified as not phototoxic.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Deltamethrin TC (AE F032640)
Description:	White solid
Lot/Batch #:	ABKBDCK008
Purity:	99.9 % w/w
CAS #:	52918-63-5
Stability of test compound:	The test material was found to be stable over a 2-year storage period
Solvent used:	EBSS (Earle's Balanced Salt Solution) containing 1% (v/v) DMSO

2. Vehicle and/or positive control: **Vehicle:** EBSS (Earle's Balanced Salt Solution) containing 1% (v/v) DMSO
Positive control: Chlorpromazine: from 6.25 to 200 µg/mL in absence of irradiation; from 0.125 to 4.0 µg/mL in presence of irradiation

3. Test Cells: BALB/c 3T3 cells clone 31 8 supplied by [redacted] Germany).

4. Culture Medium: Large stocks (Master Cell Stock) of the BALB/c 3T3 31 cell line are stored in liquid nitrogen in the cell bank [redacted]. A working cell stock is produced by multiplying from the master cell stock. Thawed stock cultures were propagated at 37 ± 1.5 °C in 75 cm^2 plastic flasks. Seeding was done with about 1×10^6 cells per flask in 15 mL of Dulbecco's Minimal Essential Medium (DMEM), supplemented with 10% newborn calf serum. The cells were sub-cultured twice weekly. The cell cultures were incubated at 37 ± 1.5 °C in a 7.5 ± 0.5% carbon dioxide atmosphere.

5. Test compound concentrations: with and without irradiation: 0.78, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100 µg/mL

6. Solar simulator: The irradiation was performed with a Dr. Hönle Sol 500 solar simulator. The filter H1 was used to keep the UVB irradiation as low as possible. The produced wavelength of the solar simulator with the filter was > 320 nm. Due to the inhomogeneous distribution of irradiation intensity the UVA intensity was measured at the complete area with a UV-meter. The homogeneous area was marked and the cultures were irradiated in this area. The solar simulator was switched on about 30 minutes prior to the start of experiment. The absorption spectrum of the test item was determined in the range from 270-800 nm. The test item showed absorption maxima in the range of 272.9 to 278.0 nm.

B. TEST PERFORMANCE

1. Seeding of the Cultures

2 x 10^4 cells per well were seeded in 100 µL culture medium (two plates, one was exposed to artificial sunlight, one was kept in the dark).

2. Treatment

24 hours after seeding the cultures were treated with the test item. The treatment was performed according to the OECD guideline as follows:

- the cultures were washed with EBSS;
- 8 dilutions of the solved test item were tested on two 96-well plates (100 µL/well);
- both plates were pre-incubated for 1 hour in the dark;

- after one hour one 96-well plate was irradiated through the lid at 2.4 – 2.55 mW/cm^2 (7.2 – 7.65 J/cm^2), for 50 ± 2 min at 20 - 30 °C, the other plate was stored for 50 ± 2 min at 20 - 30 °C in the dark;.
- after irradiation the test item was removed and both plates were washed twice with EBSS;
- fresh culture medium was added and the cells were incubated for 21 ± 3 hours at 37 ± 1.5 °C and 7.5 ± 0.5% CO2.

3. Determination of Neutral Red Uptake

The medium was removed and 0.1 mL serum free medium containing 50 µg Neutral Red / mL were added to each well. The plates were returned to the incubator for another 3 hours to allow uptake of the vital dye into the lysosomes of viable cells. Thereafter the medium was removed completely and the cells were washed with EBSS. Then 0.15 mL of a solution of 49% (v/v) deionised water, 50% (v/v) ethanol and 1% (v/v) acetic acid were added to each well to extract the dye. After additional approx. 10 min at room temperature and a brief agitation, the plates were transferred to a microplate reader (Versamax®, Molecular Devices) equipped with a 540 nm filter to determine the absorbance of the extracted dye. This absorbance showed a linear relationship with the number of surviving cells.

4. Data Recording

The data generated were recorded in the laboratory raw data file. The results are presented in tabular form, including experimental groups with the test item, solvent, and positive control. Arithmetic means ± standard deviation were calculated for every test group.

The ED_{50} values, the Photo-Irritancy-Factor (PIF), as well as the Mean Phototoxic Effect (MPE) were calculated using the software Phototox (Version 2.0) (distributed by [redacted], Germany and recommended by the OECD guideline).

The ED_{50} values (effective dose where only 50% of the cells survived) were determined by curve fitting by the software.

The PIF is defined by the following equation:

$$PIF = \frac{ED_{50}(-UV)}{ED_{50}(+UV)}$$

If a chemical is only cytotoxic +UV and is not cytotoxic when tested –UV, the PIF cannot be calculated, although this result indicates a phototoxic potential. In such cases, a > PIF value can be calculated if the (-UV) cytotoxicity test is performed up to the highest test concentration (C_{max}) and this value is used for calculation of the > PIF:

$$> PIF = \frac{C_{max}(-UV)}{ED_{50}(+UV)}$$

Since the > PIF is not an exact numerical value, no biostatistical procedure can be applied to

determine the optimum cut-off. Consequently, the classification rule has to be:

If only a > PIF can be obtained, then any value > 1 predicts a phototoxic potential.

The Mean Phototoxic Effect (MPE) is based on comparison of the complete concentration response curves. It is defined as the weighted average across a representative set of photo effect values:

$$MPE = \frac{\sum_{i=1}^{n} w_i PE_{ci}}{\sum_{i=1}^{n} w_i}$$

The photo effect (PEc) at any concentration (C) is defined as the product of the response effect (REc) and the dose effect (DEc) i.e. PEc = REc x DEc. The response effect (REc) is the difference between the responses observed in the absence and presence of light, i.e. REc = Rc (-UV) – Rc (+UV). The dose-effect is given by

$$DEc = \left| \frac{C}{C^*} - 1 \right| \quad \text{if } \frac{C}{C^*} < 1$$

$$DEc = \left| \frac{C^*}{C} - 1 \right| \quad \text{if } \frac{C}{C^*} \geq 1$$

where C* represents the equivalence concentration, i.e. the concentration at which the +UV response equals the –UV response at concentration C. If C* cannot be determined because the response values of the +UV curve are systematically higher or lower than Rc (-UV) the dose effect is set to 1. The weighting factors w_i are given by the highest response value, i.e. w_i = MAX {Ri (+UV), Ri (-UV)}. The concentration grid Ci is chosen such that the same number of points falls into each of the concentration intervals defined by the concentration values used in the experiment. The calculation of MPE is restricted to the maximum concentration value at which at least one of the two curves still exhibits a response value of at least 10%. If this maximum concentration is higher than the highest concentration used in the +UV experiment the residual part of the -UV curve is set to the response value "0". Depending on whether the MPE value is larger than a properly chosen cut-off value (MPE = 0.15) or not, the chemical is classified as phototoxic.

5. Evaluation of Results

Based on the results obtained, the test item is evaluated as follows:

If **PIF < 2 or MPE < 0.1**: no phototoxic potential predicted.
If **PIF > 2 and < 5 or MPE >0.1 and <0.15** a probable phototoxic potential is predicted.
If **PIF > 5 or MPE > 0.15** a phototoxic potential predicted

6. Acceptability of the Assay

The assay meets the acceptance criteria:

- if after irradiation with a UVA dose the cell viability of the solvent control is > 80% of non-irradiated cells.
- if for the positive control Chlorpromazine the factor (PIF) between the two ED_{50} values is > 6 and

- if the mean OD_{540} of solvent controls is > 0.4.

II. RESULTS AND DISCUSSION

Table 5.2.7-01: Treatment of BALB/c 3T3 with Deltamethrin TC in the RFE

With artificial sunlight				Without artificial sunlight			
Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control	Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control
Solvent Control	0.5898*	0.0418	100.00	**Solvent Control**	0.5456*	0.0383	100.00
0.78	0.6083	0.0212	103.13	**0.78**	0.5430	0.0159	99.53
1.56	0.5935	0.0265	100.61	**1.56**	0.5566	0.0188	102.01
3.13	0.6116	0.0157	103.69	**3.13**	0.5558	0.0168	101.86
6.25	0.6466	0.0413	109.62	**6.25**	0.5566	0.0270	102.01
12.5	0.6426	0.0439	108.95	**12.5**	0.5743	0.0458	105.26
25.0	0.6407	0.0407	108.62	**25.0**	0.5110	0.0381	93.66
50.0	0.3155	0.0694	53.49	**50.0**	0.5093	0.0437	93.34
100	0.3435	0.0390	58.23	**100**	0.3897	0.0236	71.43

* mean O.D.$_{540\ nm}$ out of 12 wells

ED_{50} values = could not be determined, since the viability of the cells was not reduced with and without irradiation.

PIF = could not be determined, since no ED_{50} values could be calculated

MPE = -0.006

Table 5.2.7-02: Treatment of BALB/c 3T3 with the positive control (Chlorpromazine) in the RFE

With artificial sunlight				Without artificial sunlight			
Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control	Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control
Solvent Control	0.5229*	0.0386	100.00	**Solvent Control**	0.6026*	0.0406	100.00
0.125	0.3210	0.0419	61.38	**6.25**	0.3498	0.0111	58.04
0.250	0.1176	0.0394	22.49	**12.50**	0.0679	0.0114	11.27
0.500	0.0570	0.0062	10.90	**25.00**	0.0523	0.0030	8.67
0.750	0.0593	0.0114	11.34	**37.50**	0.0499	0.0042	8.29
1.000	0.0539	0.0018	10.31	**50.00**	0.0502	0.0030	8.33
1.500	0.0532	0.0032	10.18	**75.00**	0.0514	0.0032	8.53
2.000	0.0533	0.0018	10.20	**100.00**	0.0482	0.0034	8.00
4.000	0.0533	0.0035	10.18	**200.00**	0.0520	0.0036	8.63

* mean O.D.$_{540\ nm}$ out of 12 wells

ED_{50} value (with artificial sunlight) = 0.15 µg/mL
ED_{50} value (without artificial sunlight) = 6.65 µg/mL
PIF = 45.70
MPE = 0.783

Table 5.2.7-03: Treatment of BALB/c 3T3 with Deltamethrin TC in the ME

With artificial sunlight				Without artificial sunlight			
Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control	Conc. [µg/mL]	O.D.$_{540\ nm}$ Mean Value	Standard Deviation	% of Solv. Control
Solvent Control	0.6160*	0.0475	100.00	**Solvent Control**	0.7496*	0.0344	100.00
0.78	0.6210	0.0349	100.80	**0.78**	0.7609	0.0324	101.51
1.56	0.6427	0.0342	104.33	**1.56**	0.7599	0.0153	101.37
3.13	0.6437	0.0133	104.49	**3.13**	0.7792	0.0270	103.95
6.25	0.6627	0.0390	107.57	**6.25**	0.7813	0.0363	104.23
12.5	0.6699	0.0276	108.74	**12.5**	0.7645	0.0293	101.99
25.0	0.6668	0.0232	108.24	**25.0**	0.7683	0.0228	102.49
50.0	0.6495	0.0231	105.44	**50.0**	0.7363	0.0180	98.23
100	0.4767	0.0296	77.38	**100**	0.6035	0.0246	80.51

* mean O.D.$_{540\ nm}$ out of 12 wells

ED_{50} values = could not be determined, since the viability of the cells was not reduced below 50%with and without irradiation

PIF = could not be determined, since no ED_{50} values could be calculated
MPE = -0.023

Table 5.2.7-04: Treatment of BALB/c 3T3 with the Positive Control (chlorpromazine) in the ME

With artificial sunlight				Without artificial sunlight			
Conc. [µg/mL]	O.D.540 nm Mean Value	Standard Deviation	% of Solv. Control	Conc. [µg/mL]	O.D.540 nm Mean Value	Standard Deviation	% of Solv. Control
Solvent Control	0.6548*	0.0684	100.00	**Solvent Control**	0.7420*	0.0390	100.00
0.125	0.5502	0.0685	84.02	**6.25**	0.7176	0.0536	96.71
0.250	0.1367	0.0945	20.87	**12.50**	0.2227	0.0555	30.01
0.500	0.0705	0.0242	10.77	**25.00**	0.0576	0.0014	7.76
0.750	0.1368	0.0982	20.89	**37.50**	0.0571	0.0038	7.70
1.000	0.0780	0.0317	11.91	**50.00**	0.0567	0.0032	7.64
1.500	0.0653	0.0060	9.97	**75.00**	0.0570	0.0031	7.68
2.000	0.0820	0.0489	12.53	**100.00**	0.0570	0.0031	7.69
4.000	0.0793	0.0255	12.11	**200.00**	0.0564	0.0026	7.60

* mean O.D.540 nm out of 12 wells

ED50 value (with artificial sunlight) = 0.18 µg/mL
ED50 value (without artificial sunlight) = 11.29 µg/mL
PIF = 64.69
MPE = 0.771

The study was performed to assess the phototoxic potential of Deltamethrin TC. The test was performed using BALB/c 3T3 cells clone 31. Two experiments were performed. The first experiment served as range finder (RFE), the second experiment (ME) was the confirming experiment.

The highest concentration used in both experiments was 100 µg/mL of the test item, dissolved in DMSO (final concentration of DMSO in EBSS culture medium was 1%).

Slight cytotoxic effects were observed after treatment of cells with the test item in the presence of irradiation with artificial sunlight in the range finding experiment. Compared to the value of the solvent control, the viability of the cells was reduced to 53.49% and to 58.23%, respectively, after exposure to the two highest test item concentrations (50.0 µg/mL and 100 µg/mL). Since the viability of the cells was not reduced below 50%, neither ED_{50}-values nor a PIF could be calculated. The resulting MPE values were -0.006 (RFE). No cytotoxic effects occurred after treatment of the cells with the test item in the absence of irradiation with artificial sunlight. Therefore, ED_{50}-values of a PIF could not be calculated again. The resulting MPE was -0.023 (ME) and thus, the test item is classified as not phototoxic.

III. CONCLUSIONS

In conclusion, it can be stated that in this study and under the experimental conditions reported, the test item Deltamethrin TC does not possess any phototoxic potential.

CA 5.3 Short-term toxicity

All necessary short term toxicity studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.
Although toxicokinetic parameters (blood concentration of glufosinate-ammonium and its main metabolites) are now required in the EU regulation 1107/2009 in most toxicological studies, these evaluations were not done for deltamethrin as the short-term toxicity studies have not been repeated recently.
Short term oral toxicity of deltamethrin was investigated in the mouse (28-day toxicity study, 12-week toxicity study), in the rat (28-day toxicity study, 90-day toxicity studies) and the dog (90-day toxicity studies, 1 year and 2 years toxicity studies). In all species, the nervous system was the main target organ with observation of different neurotoxic signs but not associated with any histopathological findings in the nervous system.
In a 90-day rat gavage study ([redacted]; 1977; M-149356-01-1), hypersensitivity was observed at 10 mg/kg/day. In a second study ([redacted]; 1991; M-149359-01-1), poor clinical conditions and neurotoxic effects were observed in both sexes at 1000 ppm and above (84 mg/kg/day). These clinical signs consisted of uncoordinated movement, unsteady gait, hunched posture, increased sensitivity to sound, piloerection, dark extremities and emaciated appearance. At 3000 and 6000 ppm, body tremors, wet dog shakes, spasmodic convulsions, semi-closed eyes, poor grooming, subdued behavior and wet urogenital fur were additionally noted. The NOAEL was set at 30 ppm (3 mg/kg/day) based on body weight gain decrease in females at 300 ppm.

In the first 90-day dog study ([redacted]; 1979; M-175845-02-1), unsteadiness, body tremors, jerking movements and increased salivation were mainly observed at the start of the study at 10 mg/kg/day. Liquid faeces, dilated pupils, depression of the flexor reflex, exaggeration or depression of the patellar reflex and modifications of the EEG (electroencephalography) were also observed at 2.5 mg/kg/day. The NOEL was set at 1 mg/kg/day. In the second 90-day study ([redacted]; 1991; M-149358-01-1), intermittent unsteady gait, body tremors, vomiting, increased salivation, shaking of the head, chewing of the extremities, quiet behavior or hunched posture were observed at the top dose of 50 mg/kg/day. The NOEL was 10 mg/kg/day. A 2-year dog dietary study was initially conducted where the animals were administered at 1, 10 or 40 ppm. No significant treatment-related findings were reported. In the one year dog study, chewing or scratching of the extremities, abnormal gait, tremors and liquid faeces were observed from 10 mg/kg/day. Vomiting, abnormal head movements, unsteadiness and incoordination of the gait and splaying of the limbs or the digits were also observed at 50 mg/kg/day. The NOAEL was 1 mg/kg/day based on slight changes in serum albumin and calcium associated with increased incidence of liquid faeces and slight decrease in red blood cell parameters from 10 mg/kg/day.

The mouse is the less sensitive species. No neurological findings were reported in the 27-day study although mortality was seen at 400 ppm ([redacted]; 1977; M-149361-01-1). In the 12-week toxicity study, clonic contractions and convulsions were observed at dose levels causing mortalities from 3000 ppm (603.4/738.8 mg:kg/day in males and females respectively) ([redacted]; 1991; M-149360-01-1).

Repeat administration of deltamethrin induced also body weight or body weight gain effects in rodents and dogs often associated with decreased food consumption.

Systemic toxicity was not observed after repeated dermal exposure. Only dermal changes due to irritation were observed. Irritation signs were also observed after repeated inhalation exposure with neurological changes observed at 9.6 and 56.3 mg/m³.

Table 5.3 -01: Summary of short-term toxicity of deltamethrin (new studies not yet submitted highlighted in black and bold – studies in the baseline dossier in gray)

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
27-day mouse study, [redacted], 1977 M-149361-01-1, 0, 200 increased to 400 ppm on W2	**not designed to identify a NOAEL**				↓ Food consumption, ↓ weight gain or even weight loss at 400 ppm Mortality at 400 ppm (5/35), ↓ liver& kidney weights without histopathological findings
28-day rat study, [redacted], 1977 M-149362-01-1, 0, 200 ppm	**not designed to identify a NOAEL**				↓ Food consumption and body weight loss during the first, no significant effect later on
90-day rat gavage study (+ 4 weeks recovery), [redacted] 1977, M-149356-01-1, 0, 0.1, 1, 2.5 and 10 mg/kg/day	**-**	**10**	**-**	**>10**	Transient hypersensitivity at 10 mg/kg/d during week 6 (observed only once during the study), ↓ weight gain in males at 2.5 and 10 mg/kg/day (less than 10%)
90-day rat dietary study, [redacted] 1991 M-149359-01-1, 0, 30, 300, 1000, 3000, 6000 ppm, 0, 2/3, 24/30, 72/84, 425/444 mg/kg/day in M/F	**NOAEL : 300 in M/F**	**24/30**	**1000**	**72/84**	Total mortality at 3000 and 6000 ppm with overt toxicity, marked neurological disturbence, 1/20 M + 2/20 F mortality at 1000 ppm with uncoordination, unsteady gait, hunched posture, ↑ sensitivity to sound, piloerection, ↓ body weight

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
					gain + food consumption during the 2 first weeks
12-week mouse dietary study, [redacted], 1991, M-149360-01-1, 0, 30, 300, 3000, 6000 ppm	300	62/77 in M/F	3000	603/739 in M/F	Mortality from 3000 ppm (3/10 M & 1/10 F) Clonic contractions in 1/10 M at 3000 ppm, clonic contractions + convulsions at 6000 ppm in most animals Slight ↓ BWG at 30 & 300 ppm
90-day dog capsule study (+ 20 weeks of recovery) [redacted] 1979, M-175845-02-1, 0, 0.1, 1, 2.5, 10 mg/kg/day	-	**NOAEL: 2.5**	-	**10**	Neurological signs mainly seen at 10 mg/kg/day (unsteadiness, body tremors, jerking movements, excessive salivation) Pupil dilation from 2.5 mg/kg/day ↓ Food consumption, ↓ weight gain during the two first weeks at 10
90-day dog capsule study (+ 4 weeks of recovery), [redacted] 1991, M-149358-01-1, 0, 2, 10, 50 mg/kg/day	10		50		Neurological signs limited to the group treated at 50 (intermittent unsteady gait, body tremors, vomiting…), ↓ Food consumption, ↓ weight gain
52-week dog capsule study, [redacted] 1993, M-149298-01-1, 0, 1, 10, 50 mg/kg/day	-	1	-	10	Unsteadiness of the gait + chewing/scratching of the extremities + liquid faeces from 10, + body tremors + bobbing or shaking of the head at 50, initial ↓ body weight gain + food consumption at 50, few biochemistry changes may be linked to liquid faeces at 10 + 50
2-year dog dietary study, [redacted] 1980, M-094407-01-1, 0, 1, 10, 40 ppm	40	1.13/1.06	>40	1.13/1.06	2 controls and 2 treated dogs died after infection +/- convulsions, few neurological signs not treatment-related – dose levels too low

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
21-day dermal study, [redacted], 1993, M-131952-01-1, 0, 100, 300, 1000 mg/kg/day in PEG400	-	1000 systemic <100 dermal	-	>1000 systemic 100 dermal	Eschar formation from 100, erythema and desquamation, thickening of the skin from 300, microscopic changes not dose-related (dermal abscesses, chronic dermatitis, epidermal necrosis, parakeratosis, ulcers…)
21-day inhalation study (14 x 6 hours exposure), [redacted] 1979, M-227755-01-1, 0, 3, 9.6, 56.3 mg/m^3	-	< 3 mg/m^3	-	3 mg/m^3	Irritation, agitated grooming, ptyalism in all groups, peripheral vasodilatation + scratching from 9.6, ataxia + walking with arched back at 56.3, serum sodium from 9. 6

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

A classification of STOT-RE is indicated when toxic effects that may include the following descriptions occur at or below 100 mg/kg/d.

Substance-related deaths: Mortality was observed at 1000 ppm (72/84 mg/kg/day in males and females, respectively) in 1/20 male and 2/20 females in a 90-day rat study, at 800 ppm (54 and 58 mg/kg/day in 3/10 males and 2/10 females) in the 90-day rat neurotoxicity study.

Major function changes in the central or peripheral nervous systems and/or other organ systems:

In the dog, transient neurological signs (including unsteadiness, body tremors, jerking movements and chewing of the extremities) were observed from 10 mg/kg/day in the first 90-day dog study and in the one year dog study and from 50 mg/kg/day in the second 90-day dog study.

In the second 90-day rat study, uncoordinated movements, unsteady gait, hunched posture and increased sensitivity to sound were observed at 1000 ppm (72/84 mg/kg/day in males and females, respectively). The incidence and severity of these findings declined from week 3 and were no longer apparent on week 8. In the rat carcinogenicity study, uncoordinated movements of the limbs characterized by splayed limbs and unsteady gait were observed from 500 ppm (22 mg/kg/day). However all these neurological effects were transient or seen only on very few occasions.

Hypersensitivity to noise, gait alterations (rocking, lurching or swaying, walking with hindlimbs splayed, walking on tiptoes), impaired righting reflex and piloerection were noted in all animals from the 800 ppm group in the 90-day rat neurotoxicity study. Convulsions, popcorn seizures and writhing were also observed in the animals which died during the study with the exception of one female.

In mice no neurological signs were observed at or below 100 mg/kg/day.

Any consistent changes in clinical biochemistry, haematology or urinalysis parameters that indicate severe organ dysfunction : no significant changes observed.

Severe organ damage noted in microscopic examination following autopsy: The histopathological findings with ballooned cells in the liver and eosinophilic hepathocytes were observed from 125 ppm (5 mg/kg/day) in the rat carcinogenicity study. However these findings were not considered severe. No other effects indicative of severe organ damage (necrosis, fibrosis, granuloma formation in vital organs with regenerative capacity; evidence of appreciable cell death in vital organs incapable of regeneration) were reported.

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

CLP regulation STOT RE2 (nervous system)

CA 5.3.1 Oral 28-day study

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.3.1/01; [redacted]; 1977; M-149361-01-1
Title:	A study of the effects of RU 22974 on food consumption in the mouse.
Report No.:	A708/7
Document No.:	M-149361-01-1
Guideline(s):	-
Guideline deviation(s):	-
GLP/GEP:	**no**

Experimental design

RU 22974 (deltamethrin) (purity not specified) was administered in the diet as a 1% premix in maize starch to 35 male OFA mice (Swiss derived) for 21 days. The dietary level of RU 22974 was 200 ppm for the first 10 days, then 400 ppm (the concentrations corresponded approximately to the theoretical doses of 28 and 57 mg/kg bw/day, respectively). The control animals (35 males) received the feed with maize starch, only. At the end of the treatment, all animals were killed and the liver and kidneys removed for histological examination.

This study was designed to evaluate the effects of certain doses on body weights and feed intake. The study fulfilled this purpose and the results are in line with dose responses in the 90-day studies.

Results

A mortality of 14% (5/35) was recorded at the end of the study in the treated animals. Comment: *These animals died during the night and were partially cannibalised. The animals were therefore not examined for histological changes.* Statistically significant reduced body weight gain, reduced final body weight, and lower food consumption (occasional statistically significant) were noted in all treated animals compared with controls.

Statistically significant reduced liver weights (absolute and relative values), and statistically significant reduced kidney weights (absolute value) were noted in the treated animals compared with the controls. The histological examination of the liver and kidneys did not reveal any evidence of lesions.

Comments from the former RMS Sweden

No NOEL for male and female mice was determined in this study. The study was conducted to evaluate the effects on food consumption and body weight of the addition of RU 22974 to the feed of laboratory mice. No other parameters were studied. No OECD guidelines exist for this type of study. The most serious shortcoming is that the same animals were used for the different dose levels. No individual animal data concerning pathological findings was presented. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). However, the study seems to be of acceptable quality.

Report:	KCA 5.3.1/02; [redacted]; 1977; M-149362-01-1
Title:	RU 22974: Study of the effects of RU 22974 on food consumption in the rat
Report No.:	A70878
Document No.:	M-149362-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

RU 22974 (deltamethrin) (purity not specified) was administered in the diet as a 1% premix in maize starch to 10 male OFA rats (Sprague Dawley derived) for 4 weeks. The dietary level of RU 22974 was 200 ppm (the concentration corresponded approximately to the theoretical dose of 13 mg/kg bw/day). The control animals (10 males) received the feed with maize starch only.

This study was designed to evaluate the dose response of different doses on body weights and feed intake. The study fulfilled this purpose and results are in line with dose responses in the 90-day studies.

Results

There were no deaths during the study. Body weights were lower in the treated animals than in the control animals (statistically significant at 4 and 7 days). Statistically significant reduced food consumption was seen in the treated animals during the first week of treatment.

Comments from the former RMS Sweden

No NOEL for male and female rats was determined in this study. The study was conducted to evaluate the effects on food consumption and body weight of the addition of RU 22974 to the feed of laboratory rats. No other parameters were studied. No OECD guidelines exist for this type of study. No histological examination was performed. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). However, the study seems to be of acceptable quality.

CA 5.3.2 Oral 90-day study

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter. For some studies where neurological signs were observed, more details are provided.

Report:	KCA 5.3.1/03; [redacted]; 1977; M-149356-01-1
Title:	RU 22974: Assessment of toxicity to rats by oral administration for 13 weeks (followed by a 4-week withdrawal period).
Report No.:	A70872
Document No.:	M-149356-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

RU 22974 deltamethrin (purity not specified) was administered by oral gavage as a solution in polyethylene glycol 200 (PEG 200) to groups (20 animals/sex/group) of CD rats (Sprague-Dawley) for 13 weeks at respective doses of 0.1, 1.0, 2.5 and 10 mg/kg bw/day. The control animals (20 animals/sex) received the vehicle only. Before treatment began, and during weeks 6 and 13, ten males and ten females from the control group and from the highest dose level group were subjected to a neurological examination (segmental reflexes, postural reactions, locomotor system and general observations were studied). At termination of the study five males and five females from each group were observed for reversibility, persistence or delayed occurrence of toxic effects for a recovery period of 4 weeks.

Almost all of the required parameters in OECD guideline 408 (21st September 1998) are covered in this study with regard to animal number, recovery phase, intervals of clinical observations, determinations of body weight, food consumption, water uptake and ophthalmoscopic examinations. A declaration of the study director that the work was performed under his supervision, according to the procedures described, and that the report provides a correct and faithful record of the results obtained indicates that the study was performed in the spirit of GLP. At the highest dose clinical signs, i.e. hypersensitivity in both sexes and decreased body weight gains in males at 2.5 and 10 mg/kg bw were seen. In this study also neurological observations were performed which are similar to the ones in the modern neurotoxicity battery according to EPA. All haematological and clinical chemistry parameters are covered and even at more time-points than required in OECD 408. Also an optional urinalysis with all parameters was done. Organ weights are covered except thyroids and epididymides, which were measured in the 2-year rat studies and not affected. Histopathology was performed in the control and high-dose group and almost all required organs except aorta, trachea and mammary gland were examined. Latter organs were examined in the 2-year rat studies and found to be unaffected.

Results

There were no treatment-related deaths during the study. Slight hypersensitivity was noted in 3/10 female and 9/10 male rats receiving 10 mg/kg bw/day at week 6. The behaviour of these rats was normal by week 13. Neurological examination did not give any indication of treatment-related interference with any reflexes.

Statistically significant lower body weight gain (-8% and -9% compared to control mean) was noted in males receiving deltamethrin at doses of 2.5 and 10 mg/kg bw/day, respectively. During the recovery period, the rate of body weight gain among these rats was marginally higher than that of controls.

There were no effects on parameters concerning haematology, clinical chemistry, urinalysis or ophtalmoscopy. There were no substance-related effects on any organ weights. No substance-related gross- or microscopic changes were observed.

Comments from the former RMS Sweden

The NOEL was 1 mg/kg bw/day for male rats based on reduced body weight gain noted in males receiving deltamethrin at 2.5 and 10 mg/kg bw/day. Additionally, clinical signs (hypersensitivity) were noted for males receiving deltamethrin at 10 mg/kg bw/day. The NOEL was 2.5 mg/kg bw/day for female rats based on clinical signs (hypersensitivity) noted for females receiving deltamethrin at 10 mg/kg bw/day. The study follows OECD guideline nr 408 except for the fact that the choice of the highest dose level was too low, which restricts the sensitivity of the test. The abscence of reduced body weight gain in females indicates that the highest dose level was too low. Another shortcoming is that the age of the animals or the purity of the test substance was not specified in the study. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). Although the sensitivity of the study was restricted, the study brings some information about the expected oral short-term toxicity of deltamethrin in rats. The results of the study were therefore taken into consideration in this report.

Conclusion from the applicant:

Considering that hypersensitivity was only observed on week 6 in male rats and a few females treated at 10 mg/kg/day and only slight (-8% to -9%) lower body weight gain compared to control mean was observed in males at 2.5 and 10 mg/kg/day, the NOAEL is considered to be 10 mg/kg/day in both males and females.

Report:	KCA 5.3.2/01; [redacted]; 1979; M-175845-02-1
Title:	RU22974. Oral toxicity study in beagle dogs
Report No.:	A98072
Document No.:	M-175845-02-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

RU 22974 (deltamethrin) (purity not specified) dissolved in polyethylene glycol (PEG) 200 was administered orally (by gelatine capsule) to groups of 15 to 35- week old beagle dogs (the control and low level dose groups contained 3 male and 3 female dogs, and the remaining three groups consisted of 5 male and 5 female dogs) for 13 weeks with a recovery period for 2 male and 2 female dogs from the three highest dosage groups to a total of 20 weeks. The dose levels were 0 (vehicle control), 0.1, 1.0, 2.5 and 10 mg/kg bw/day. Neurological examinations were conducted on all dogs before dosing commenced, after 5 weeks dosing and after 12 weeks dosing. Animals which retained undosed were examined during the recovery period.

The study follows the OECD guideline 409 except that only 3 males and 3 females instead of at least 4 were used in each group.The age of the animals is not mentioned. Intervals of clinical observations, determinations of body weight, food consumption, water uptake, ophthalmoscopic examinations, haematological and urinalysis determinations comply with the guideline. Less clinical biochemistry parameters than usually suggested were determined and epididymides were not weighed. The full list of organs were examined histologically. A declaration of the study director that the work was performed under his supervision, according to the procedures described, and that the report provides a correct and faithful record of the results obtained indicates that the study was performe in the spirit of GLP.

Results

There were no deaths during the study. Unsteadiness, body tremors, jerking movements, vomiting and excessive salivation were noted in male and female dogs receiving 10 mg/kg bw/day. Liquid faeces were noted in all groups but occurred more frequently at dose levels of 2.5 and 10 mg/kg bw/day. Signs of dilation of the pupils were also seen in male and female dogs receiving 2.5 and 10 mg/kg bw/day. After the cessation of dosing, the only clinical sign observed was isolated incidences of liquid faeces in all recovery groups.

Statistically significant decreased body weight gain and improvement in appetite were noted during the first 1-2 weeks of dosing in most animals receiving 10 mg/kg bw/day. During the recovery period the changes in body weight and food consumption remained similar to that established during the dosing period.

The neurological examination showed effects upon the gag reflex (depression), the patellar reflex (hyperactivity or depression) and flexor reflex (depression). Qualitatively similar findings were also noted among the control animals. The incidence was increased among treated animals compared to the controls, although no clear dose effect could be established. The incidence was higher at the beginning of the study compared to the latter part. By the end of the recovery period, some dogs from the low level dosage groups continued to show depression of the patellar- and the gag reflex, but none of these animals were from the high dosage level groups. One dog that had received 10 mg/kg bw/day still showed depression of the flexor reflex at the end of the recovery period. The neurological changes seen in the remaining dogs had been reversed following cessation of dosing. Electroencephalograms (EEG) showed abnormal patterns in some dogs receiving 2.5 or 10 mg/kg bw/day. These abnormalities were confined almost exclusively to the occipital leads and showed persistent high amplitude, fast frequencies, often with spikes. After 5 weeks recovery one animal which had received 10 mg/kg bw/day showed abnormal spiking in all leads. No other abnormalities were seen in any recovery animals.

There were no treatment-related effects on parameters concerning haematology, biochemistry, urinalysis or ophtalmoscopy.

There were no treatment-related effects on any organ weights.

No treatment-related gross-or microscopic changes were detected in the nervous system of any dog.

Comments from the former RMS Sweden

The NOEL was 1 mg/kg bw/day for male and female dogs based on liquid faeces and dilation of the pupils noted in animals receiving deltamethrin at 2.5 mg/kg bw/day. Additionally, clinical signs (neurological effects), decreased body weight gain and decreased food consumption were noted at 10 mg/kg bw/day. The relevance of the effects upon segmental reflexes were considered equivocal and of minor toxicological importance (personal communication with expert [redacted] Sweden) due to the fact that the incidence and the variable response in the dog study as well as the absence of a control recovery group makes it difficult to clearly deem them as primary findings induced by the treatment. The overall effects upon the general condition may at least partly play a role. Furthermore, it was impossible to repeat the findings in a later performed study on the dogs where the dose levels were equal or higher compared to this study (see [redacted] 1991). The relevance of the effects upon the EEG alterations were also considered equivocal and of minor toxicological importance {personal communication with expert [redacted] Sweden), They must not necessarily be considered as direct cerebral or cerebellar effects but may as well be secondary to an increase in peripheral muscular tonic activity. There were no clinical signs attributable to these changes and the extensive histomorphological investigation of the brain and upper spinal cord showed no abnormal findings. The study follows OECD guideline no 409 with exception of the low number of animals in control- and low level dosage groups. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed) but the study is well reported and seems to be of acceptable quality.

Conclusion from the applicant:

The NOAEL is considered to be 2.5 mg/kg/day based on the neurological signs (unsteadiness, body tremors and jerking movements observed at 10 mg/kg/day and pupil dilation in 4/5 males and all females on several occasions). Pupil dilation was also observed at 2.5 mg/kg/day but in only 3/5 males and females on very few occasions.

Report:	KCA 5.3.2/02; [redacted]; 1991; M-149358-01-1
Title:	Deltamethrin: Oral toxicity study in beagle dogs (repeated dosage for 13 weeks with a 4-week recovery period).
Report No.:	A70874
Document No.:	M-149358-01-1
Guideline(s):	OECD: 409; USEPA (=EPA): 82-1
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 98.9%) was administered orally (by gelatine capsule) to groups of 25 to 28-week old beagle dogs (the control and high dosage level groups contained 6 male and 6 female animals, the remaining groups consisted of 3 male and 3 female animals) for 13 weeks with a recovery period for 3 dogs/sex from the control and high level dosage groups to a total of 17 weeks. The dose levels were 0 (control), 2, 10 and 50 mg/kg bw/day. The control animals received empty gelatine capsules. A full neurological examination covering cranial nerves, segmental reflexes and postural reactions was performed for all animals before dosing commenced, and again for all maximum dose level and control dogs at the end of the dosing period.

The study follows the OECD guideline 409 except that only 3 males and 3 females instead of at least 4 were used in each group and epididymides were not weighed.

Results

There were no deaths during the study. Treatment-related clinical signs were noted in male and female dogs receiving deltamethrin at 50 mg/kg bw/day (unsteady gait, trembling, increased incidence of vomiting, salivation, shaking of the head, chewing of the extremities, quiet behaviour and hunched posture). No treatment-related clinical signs were observed in animals receiving deltamethrin at 2 or 10 mg/kg bw/day. No signs attributable to previous treatment with deltamethrin were observed during the recovery period.

Statistically significant reduced body weight gain was observed for male dogs receiving 50 mg/kg bw/day. Statistically significant reduction on food intake was observed for male and female dogs receiving 50 mg/kg bw/day. No changes in the pattern of body weight performance that could be attributed to previous treatment were seen amongst dogs maintained for the four-week recovery period. Food intake was maximal for all dogs maintained for the 4 week recovery period.

There were no treatment-related effects on parameters concerning haematology, biochemistry, urinalysis or ophtalmoscopy.

Neurological examination showed no treatment-related findings. There was no indication of treatment-related interference with any reflexes.

Examination of bone marrow smears at termination, post mortem organ weights and macroscopic and microscopic pathology did not reveal any treatment-related changes.

Comments from the former RMS Sweden

The NOEL was 10 mg/kg bw/day for male and female dogs based on clinical signs (unsteady gait, trembling, vomiting, salivation, shaking of the head, chewing of the extremities, quiet behaviour and hunched posture), reduced food consumption and reduced body weight gain (males only) noted in animals receiving deltamethrin at 50 mg/kg bw/day. The study follows OECD guideline no 409 with exception of the low number of animals in the low- and intermediate level dosage groups. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections, and seems to be of acceptable quality.

Report:	KCA 5.3.2/03; [redacted]; 1993; M-149298-01-1
Title:	Deltamethrin (technical): Toxicity to dogs by repeated oral administration for 52 weeks.
Report No.:	A70808
Document No.:	M-149298-01-1
Guideline(s):	OECD: 452; USEPA (=EPA): 83-1 (Nov.1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Beagle dogs (4 animals/sex/group) were orally administered deltamethrin (purity 98.9%) by gelatin capsule preparations at dose levels of 1, 10 or 50 mg/kg bw/day for 52 weeks. The control (4 animals/sex) received empty gelatine capsules only. A full neurological examination covering cranial nerves, segmental reflexes and postural reactions was performed for all animals before dosing commenced and again for all high dose level and control dogs during weeks 26 and 52 of dosing.

This study is following the OECD guideline 452, revised in 2009. Four males and four females were allocated to each group and the epididymides were weighed at necropsy.

Results

There were no deaths. Clinical signs such as unsteadiness of the gait, splayed limbs/digits, chewing/scratching of the extremities and tremor and liquid faces were seen in dogs receiving deltamethrin at 10 and 50 mg/kg bw/day. Additionally, splayed limbs/digits, abnormal head movements, significant neurological impairment including inability to stand/walk and vomiting were noted in dogs at 50 mg/kg bw/day.

A dosage-related statistically significant reduction in body weight was observed for all treated males over the dosing period. Comment: *Weight gain of male controls in the study was higher than expected after comparison with historical control data and weight gain of male dogs at 1 and 10 mg/kg bw/day was within the expected background range for dogs of this strain, source and age.* A transient reduction in weight gain (not statistically significant) was observed over part of the dosing period (weeks 20 to 40) for female dogs receiving deltamethrin at 10 and 50 mg/kg bw/day. Overall food intake was reduced (statistically significant) for males receiving deltamethrin at 50 mg/kg bw/day, and occasionally reduced for individual female animals receiving deltamethrin at 50 mg/kg bw/day.

Slight reductions in red cell parameters (PCV, Hb) were noted in all males at week 26 (not statistically significant) and at week 52 (statistically significant) amongst treated males receiving deltamethrin at 10 and 50 mg/kg bw/day.

Slight decreases in serum albumin and calcium levels (not statistically significant) were noted for treated males receiving deltamethrin at 10 and 50 mg/kg bw/day. Decreased serum sodium, urea and creatinine levels were noted for males receiving deltamethrin at 50 mg/kg bw/day at week 52. These

changes were by the author considered likely to be associated with the increased incidence of liquid faeces and reduced body weight gain at this dosage.

There were no treatment-related effects on any organ weights.

There were no treatment-related effects on parameters concerning urinalysis or ophtalmoscopy. Neurological examination showed no treatment-related findings. There was no indication of treatment-related interference with any reflexes.

No treatment-related gross-or microscopic changes were observed.

Comments from the former RMS Sweden

The NOEL for female dogs was 1 mg/kg bw/day based on clinical signs (unsteadiness of the gait, chewing/scratching of the extremities, tremor, splayed limbs/digits) and liquid faeces noted in females receiving deltamethrin at 10 mg/kg bw/day. Additionally, reduced food consumption were noted for females (occasional statistically significant) receiving deltamethrin at 50 mg/kg bw/day. The NOAEL for male dogs was 1 mg/kg bw/day based on clinical signs (unsteadiness of the gait, chewing/scratching of the extremities, tremor, splayed limbs/digits) noted in males receiving deltamethrin at 10 mg/kg bw/day. Minor changes in haematological parameters (reduced PCV, Hb) were also noted in males receiving 10 mg/kg bw/day, and reduced body weight gain and food consumption were noted in males receiving deltamethrin at 50 mg/kg bw/day. The dosage-related trend in the body weight gain reduction amongst males, extending to low dose level (1 mg/kg bw/day) suggest that this change may possibly be related to treatment for all groups of treated males. However, as the growth of dogs, especially in the low dosage group, was within expected limits and was in fact in excess of that recorded for some groups of comparable historic control animals used in the same laboratory ([redacted]), 1 mg/kg bw/day was considered a NOAEL level for deltamethrin in the beagle dog. The study follows OECD guideline no 409 except for the fact that the age of the animals was not specified. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality. *This study served as basis for calculation of the proposed ADI and AOELfor deltamethrin.*

Report:	KCA 5.3.2/04; [redacted] 1980; M-094407-01-1
Title:	2-Year Chronic Dog Feeding Study
Report No.:	A21228
Document No.:	M-094407-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (RU 22974) (purity not specified) was suspended in corn oil and administered in the diet to groups (8 animals/sex/group) of beagle dogs (3 to 4-months old) at respective concentrations of 1, 10 and 40 ppm for 2 years (the concentrations corresponded to a mean calculated daily intake of

0.03, 0.26 and 1.13 mg/kg bw/day for males and 0.02, 0.27 and 0.98 mg/kg bw/day for females). The controls (8 animals/sex) received corn oil only. Neurological examinations were conducted at approximately 1 year and before termination (cranial nerves, segmental reflexes and postural reactions were investigated).

This study is following the OECD guideline 452, revised in 2009, except that the epididymides, the uterus and the thymus were weighed at necropsy.

Results

There were no treatment-related deaths during the study (two control dogs and two treated dogs at the 1- and 10-ppm dosage level groups died during the study attributed to an infectious process of unknown etiology). No signs of overt toxicity were observed in any of the treated dogs. Incidence of soft stool/diarrhea was high for the treated dogs. Comment: *There are no data to confirm this statement.*

Body weights and food consumption values were similar for control and treated dogs.

No compound-related effects were observed during the ophthalmoscopic and physical examination. Comment: *Although there were some random statistically significant differences between the control and other dose groups in the hematologic and biochemical tests, there were not any physiologically significant changes observed at any interval in this study.*

Statistically significant increased mean spleen weight (relative value) was noted in males fed deltamethrin at 40 ppm at terminal sacrifice. Neurological examination did not give any indication of treatment related interferences with any reflexes.

No compound-related gross- or microscopic changes were observed.

Comments from the former RMS Sweden

The NOAEL for male dogs and the NOAEL for female dogs was >40 ppm (1 mg/kg bw/day for males and females). No signs of toxicity on deltamethrin were observed in this study except for increased mean spleen weight noted in male dogs fed deltamethrin at 40 ppm. The study follows OECD guideline no 452 except for the fact that the choice of the dose levels were too low. This fact severely restricts the sensitivity of the test. In short term oral toxicity studies on dog, dose levels up to 50 mg/kg bw/day were used (see table B.5.24). Haematological examination were conducted on all dogs twice during the pretesting period and at 6, 12 and 24 months (according to the OECD guideline no 452, haematological examination should be performed at 3 months, 6 months and at approximately 6 month intervals thereafter and at termination). The purity of the test substance was not specified. There are no statements concerning GLP but the study was subjected to Quality Assurance inspection (GLP was not compulsory at the time when this study was performed). The study is not of acceptable quality due to the unacceptable low dose levels used.

Report:	KCA 5.3.2/05; [redacted]; 1991; M-149359-01-1
Title:	Deltamethrin toxicity studies in rat by dietary administration for 13 weeks with a 4-week recovery period (3 volumes).
Report No.:	A70875
Document No.:	M-149359-01-1
Guideline(s):	MAFF: 59 Nohsan No.4200, (Jan.1985); OECD: 408, (Aug.1981); USEPA (=EPA): F, 82-1, (Nov.1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 98.9%) was administered by admixture with the diet to rats of the Crl:CD (SD) BR strain (20 animals/sex/group) at concentrations of 0, 300, 3000 and 6000 ppm for 13 weeks. The concentrations corresponded to a dose rate of 0, 24, 241 and 425 mg/kg bw/day for males, and 3, 30, 272 and 444 mg/kg bw/day for females. The control animals (20 animals/sex) received the feed only. A supplementary study (20 animals/sex/group) was commenced at a dietary inclusion level of 0 and 1000 ppm following premature sacrifice of rats receiving 3000 and 6000 ppm due to severe reaction to treatment in the initial study. The concentrations corresponded to 0 and 72 mg/kg bw/day for males, and 0 and 84 mg/kg bw/day for females. Selected animals (10 rats/sex/group) from the inital and supplementary study groups were retained for a further 4-week recovery period and given untreated diets.

This study follows the OECD 408 version before 1998 and was performed under GLP. The animals numbers in the groups were in agreement with OECD 408 (1998), this is also valid for the time points of the clinical observations, of the body weight and feed intake determinations and the ophthalmoscopic examination. No determination of grip strength and motor activity was performed, however, this was covered by the 90-day rat study of [redacted]; 1977; M-149356-01-1 and the neurotoxicity rat studies. The laboratory investigations (haematology, clinical chemistry, urinalysis) are in agreement with the current OECD 408. The required organ weight measurements were done with the exception of epididymides and thymus, however thyroids and pituitary weight were determined although not required by the current OECD 408. Epididymal and thymus weights were determined in the 2-year rat studies in which no changes were seen. The histopathological investigations in the control and 1000 ppm group were in agreement with the current OECD 408, only stomach and oesophagus were not examined which, however was done in the 2-year rat studies in which no changes occurred in these tissues.

Results

All rats receiving 3000 or 6000 ppm, and 3 rats (2 females and 1 male) receiving 1000 ppm were either found dead or killed in extremis due to severe reaction to treatment during the first 3 weeks of treatment. Animals treated at 1000 ppm showed uncoordinated movement, unsteady gait, hunched posture, increased sensitivity to sound, piloerection, dark extremities and emaciated appearance. Body tremors, "wet dog shakes", spasmodic convulsions, semi-closed eyes, poor grooming, subdued behaviour, wet urogenital fur and emaciation were additionally noted in

animals treated at 3000 and 6000 ppm. The incidence and severity of the clinical signs among animals receiving 1000 ppm declined from week 3 of treatment and, on the whole were no longer apparent after 8 weeks of treatment. No clinical signs considered to be related to previous treatment were noted during the recovery period in both studies.

Statistically significant reduced body weights were noted for males and females receiving 3000 and 6000 ppm. Statistically significant reduced bodyweight gain was noted for females receiving 30 and 300 ppm (-15% in both groups at the end of the first week of treatment compared to control mean and -14.5% and -9% in the 30 and 300 ppm treated groups, respectively at the end of the second week), and for males and females receiving 1000 ppm (body weight loss in the males at the end of the first week and -20% compared to the control mean for the two first weeks, for the females: -92% and -72% compared to control mean at the end of the first week or the two first weeks, respectively). During the recovery period, bodyweight gain was marginally greater for animals previously treated with 1000 ppm deltamethrin in comparison with concurrent controls. Food consumption and water intake was statistically significantly reduced for animals receiving 1000, 3000 and 6000 ppm. During the recovery period food consumption for females previously treated with 1000 ppm deltamethrin was marginally greater than that of concurrent controls, and food intake for males previously treated at this same dosage level became similar to that of controls.

Table 5.3.2 -01: Summary of group mean body weight gain (g/rat)

Study intervals in weeks	DLT dose levels in ppm						
	Initial groups					Additional groups:	
	0	30	300	3000	6000	0	1000
Males							
0 -1	54.9	53.5	52.6	-12.6**	-24.6**	60.4	-2.8**
0 - 2	104.6	106.4	106.1	5.5 **	-	111.9	21.9**
0 - 13	389.2	366.2	401.8	-	-	358.4	288.0**
13 - 17	30.4	16.9	26.3	-	-	18.3	24.7
Females							
0 -1	32.8	27.9*	27.8*	-2.3**	-16.3**	34.9	2.9**
0 - 2	58.3	49.9	53.1	8.1**	-	59.5	16.8**
0 - 13	173.9	156.0*	152.3**	-	-	164.5	143.2**
13 - 17	5.9	5.9	9.1	-	-	3.7	8.5

There were no treatment-related effects concerning ophtalmoscopy, hematology, biochemistry or urinalysis parameters.

There were no substance-related effects on any organ weights. No substance-related gross- or microscopic changes were observed.

Comments from the former RMS Sweden

No NOEL was determined for females in this study due to reduced body weight gain in females receiving deltamethrin at 30 ppm. Additionally, deaths, clinical signs (poor clinical condition and neurological disturbances), reduced food consumption and decreased water intake were noted in females receiving deltamethrin at 1000 ppm. The NOEL for male rats was 300 ppm based on death, clinical signs (poor clinical condition and neurological disturbances), reduced body weight gain, decreased food consumption and decreased water intake noted in males receiving deltamethrin at 1000 ppm. The study follows OECD guideline no 408. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Conclusion from the applicant:

As only slight body weight gain decrease was observed in the females treated at 30 and 300 ppm, the NOAEL is considered to be 300 ppm for both sexes (equating to 24 and 30 mg/kg/day in males and female, respectively) based on mortality and neurological clinical signs observed at 1000 ppm (equating to 72 and 84 mg/kg/day in males and females, respectively).

Report:	KCA 5.3.2/06; [redacted]; 1991; M-149360-01-1
Title:	Toxicity study for 12 weeks by oral administration to mice.
Report No.:	A70876
Document No.:	M-149360-01-1
Guideline(s):	USEPA (=EPA): F, 82-1, (Nov.1984)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 99.7%) was administered in the diet to groups (10 animals/sex/group) of Swiss mice from the strain CD 1 Crl CD-I BR at respective concentrations of 30, 300, 3000 and 6000 ppm for 12 weeks. The concentrations corresponded to a dose rate of 6, 52, 603, 1318 mg/kg bw/day for males and 8, 77, 739, 1391 mg/kg bw/day for females. The control animals (10 animals/sex) received the feed only. Satellite groups composed of 5 males and 5 females each were administered diet containing 30 and 6000 ppm deltamethrin for possible determination of plasma levels.

This study was designed as a dose range finding study to determine doses for the mouse oncogenicity study and not as a study from which an endpoint should be derived, therefore, a strict agreement with OECD 408 is not obligatory. Nevertheless, this study almost followed for all parameters the current OECD 408 guideline, like the time points of the clinical observations, of the body weight and feed intake determinations. Ophthalmoscopic examination were not peformed. Most of the haematological (except clotting time) and the clinical chemistry (except sodium, potassium, protein and albumin) parameters are covered and the missing parameters were investigated in rat studies. The organ weights did not cover epididymides, uterus, thymus, brain and heart, but these are covered by many other studies. Histopathological examination of all required organs per guideline was performed in the control and 3000 and 6000 ppm groups.

Results

Mortality occurred at 3000 ppm (3/10 males and 1/10 females) and at 6000 ppm (14/15 males and 14/15 females) versus 1/10 males and 1/10 females in the control *group*. Clinical signs of poor condition (piloerection, dyspnea and arched back) were observed in males receiving deltamethrin at 3000 and 6000 ppm, and in females receiving deltamethrin at 6000 ppm. Additional, convulsions were observed in both males and females receiving deltamethrin at 6000 ppm. Clonic contractions were noted in males receiving deltamethrin at 3000 and 6000 ppm, and in females receiving deltamethrin at 6000 ppm A decrease in body weight gain (statistically significant) was observed in comparison with controls for the 30 to 3000- ppm male dosage levels (differences at the end of the treatment period of 8% at 30 and 300 ppm, and 14% at 3000 ppm). Statistically significant decreased body weight was observed in comparison with controls for the 3000 ppm- female and 6000 ppm- male dosage levels (group mean body weight differences of 18% at 6000 ppm and 11% at 3000 ppm). Food consumption was decreased for males and females receiving deltamethrin at 3000 and 6000 ppm.

There were no treatment-related effects on parameters concerning haematology or blood biochemistry. There were no substance-related effects on any organ weights. No treatment-related gross microscopic changes were observed.

Thymic involution (lymphoid depletion) and lipid depletion in the adrenal glands were observed in animals at 3000 and 6000 ppm which were found dead. Comment: *According to the author they could be indicative of stress phenomenon which may be secondary to the poor physical condition of these animals before death.*

Comments from the former RMS Sweden

No NOEL was determined for male mice due to slight decrease in body weight gain in males receiving deltamethrin at 30 ppm. Additionally, deaths, clinical signs (poor condition, clonic contractions) and decreased food consumption were noted in males at 3000 ppm and decreased body weight was observed in males at 6000 ppm. NOEL was 300 ppm for female mice based on death, decreased body weight and decreased food consumption at 3000 ppm. Additionally clinical signs (poor condition, convulsions, clonic contractions) were noted in females at 6000 ppm. The study follows OECD guideline no 408 except for the fact that no additionally satellite group of animals treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects was used in the study for 28 days post treatment. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance Inspections, and seems to be of acceptable quality.

Conclusion from the applicant:

As only slight body weight gain decrease was observed in the males treated at 30 and 300 ppm, the NOAEL is considered to be 300 ppm for both sexes (equating to 62 and 77 mg/kg/day in males and female, respectively) based on mortality and neurological clinical signs observed at 3000 ppm (equating to 603 and 739 mg/kg/day in males and females, respectively).

CA 5.3.3 Other routes

All necessary toxicity studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.3.3/01; [redacted]/1993; M-131952-01-1
Title:	21-day dermal toxicity study in rats with deltamethrin technical.
Report No.:	A50968
Document No.:	M-131952-01-1
Guideline(s):	USEPA (=EPA): 82-2
Guideline deviation(s):	-
GLP/GEP:	**yes**

Experimental design

Groups of five male and five female rats (Sprague Dawley [redacted]) were dermally administered deltamethrin (purity 99.6%) for three weeks at respective doses of 100, 300 and 1000 mg/kg bw/day. The test article was mixed with polyethylene glycol 400 (PEG 400). The control animals (5 animals/sex) received the vehicle only. A complete gross necropsy examination was performed on all animals. The liver and kidneys from control and high-dose animals, and the treated skin, untreated skin and gross lesions from all animals were examined microscopically.

This study followed OECD guideline 410 with regard to animal numbers, dose levels, clinical and dermal examinations, treatment duration, body weight and feed intake determination intervals. A recovery group which is optionel according to this guideline was not included. The required haematology and clinical chemistry parameters were covered, also all organ weights (except adrenals) and organs which were examined histopathologically.

Results

No mortality or clinical signs of toxicity were observed during the study.

Mean body weight gain and food consumption appeared to be slightly decreased for males of the 300 and 1000 mg/kg bw/day groups (not statistically significant).

There were no treatment-related effects on clinical pathology, necropsy or organ weight data.

Signs of dermal irritation were observed in animals in all treated groups. Eschar were observed in 3/5 males at 100 mg/kg/day. In the 300 and 1000 mg/kg/day groups, dermal findings included slight erythema, slight desquamation, eschar formation, eschar exfoliation and thickening of the skin. Substance-related microscopic dermal changes in these groups included dermal abscesses, chronic dermatitis, exudate on the epidermal surface, mononuclear cell foci in the dermis, epidermal necrosis, parakeratosis, ulcers and epidermal vesiculation.

Comments from the former RMS Sweden

No NOEL for male and female rats was determined in this study. Signs of dermal irritation were observed in all animals dermally exposed to deltamethrin. The study follows OECD guideline no 410 except for the fact that no additional satellite group of animals treated with the high dose level for 21 days and observed for reversibility, persistence or delayed occurrence of toxic effects for 14 days post-treatment was used in the study. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report: KCA 5.3.3/02; [redacted]; 1979; M-227755-01-1

Title: RU22974 (Decis) 3 week inhalation toxicity study in rats
Report No.: A95071
Document No.: M-227755-01-1
Guideline(s): -
Guideline deviation(s): -
GLP/GEP: **no**

Experimental design

Groups of eight male and eight female albino rats [redacted] were exposed whole body to dust aerosol atmospheres of deltamethrin (purity not specified) at respective concentrations of 3, 10 and 56 mg/1,6 m a day, 5 days a week for 2 weeks and 4 days on the 3rd week (a total of 14 exposures). The controls (8 animals/sex) received air only. The average percentage of particulate deltamethrin considered respirable (5.5 nm mean aerodynamic diameter) was about 87%.

With the exception of the study duration this study covers many requirements of OECD guideline 412 (7 Sept. 2009). Also characterization of exposure conditions, like measurement of aerosol concentrations and particle size distributions were performed, the daily exposure time of 6 hours is in agreement with OECD 412. Minor deviations in temperature and relative humidity are not expected to interfere with the results. Relevant organ weights (except lung) were taken and all required organs and tissues were examined histopathologically. GLP conditions are not reported, but overall this study has a high quality and the study results are a solid basis for conclusions on the inhalation toxic potential.

Results

All rats survived to necropsy. Rats exposed to the dust of deltamethrin showed symptoms as licking of the inside of the mouth, blinking, washing and scratching of the face and skin, and ptyalism. Hypersensitivity, aggressive behaviour, ataxia and walking with arched backs were observed in rats exposed at the high dose level. All rats became normal between exposures.

Statistically significant reduced body weight gain was noted in male rats in all three groups exposed to the dust of deltamethrin. Compared to control values, lower amounts of food were consumed by rats in all three groups exposed to the dust of deltamethrin.

Statistically significant increases above control values were calculated for serum sodium values in both male and female rats in the intermediate- and high dose groups. An increased high group mean urea concentration was obtained for male rats in the high dose group.

Increases ($p<0.05$) in organ weights (relative values) occurred in adrenal (males) at 56 mg/1. Decreases ($p<0.05$) in organ weights (relative values) occurred in heart (females) at 56 mg/1.

No treatment-related gross or microscopic changes were observed except for scarring of the ears in rats exposed at the intermediate- and high dosage levels. This effect was considered indirectly related to the irritant nature of deltamethrin.

Comments from the former RMS Sweden

No NOEL for male and female rats was determined in this study. Clinical signs (irritative and neurological effects) were seen in all rats exposed to the dust of deltamethrin. The study follows OECD guideline no 412 with exception of some minor deviations. The temperature in the exposure chamber varied between 21-29°C and the humidity varied between 13-89% (recommended values according to the OECD guideline no 412 are 22±3°C and 30-70% humidity). The study did not include any satellite group of animals, treated with the high dose level for 14 days and observed for reversibility, persistence or delayed occurence of toxic effects for 14 days post-treatment. The purity of the test substance was not specified. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). However, the study seems to be of acceptable quality.

Conclusion from the applicant:

NOAEL approximately 3 mg/kg/day as the clinical signs observed in this group were limited (licking of the inside of the mouth, blinking and increased grooming behaviour, ptyalism in one male noted once.

CA 5.4 Genotoxicity testing

Deltamethrin has no genototoxic potential as previously demonstrated in a full battery of *in vitro* or *in vivo* tests. Three new *in vitro* genotoxicity studies including an new Ames test, an *in vitro* micronucleus test and and HPRT test have been performed on the current specification. A detailed summary for each of these studies is presented below. For the genotoxicity studies reviewed during previous submission, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

The new Ames test was performed in 2005 at the request of the Japanese authorities ([redacted] 2005; M-253266-01-2). The test substance, Deltamethrin (Max. conc. 5000 µg/plate, batch supported the current specification) was investigated in reverse mutation test with and without metabolic activation using Salmonella typhimurium TA strains (TA98, TA100, TA1535, TA1537) as well as Escherichia coli WP2uvrA/pKM101 strain. Dose-dependent increases of revertant colonies were not observed for any strains with or without metabolic activation. The growth inhibition was not observed at up to 5000 µg/plate in any strain. The precipitation on plates was noted at 5000 µg/plate. On the other hand, in application of the positive control substances (AF-2, NaNs, 9-AA, 2-AA), a marked increase of revertant colonies for all test strains was observed, indicating that this test was performed under the appropriate conditions. Therefore, it was concluded that test substance Deltamethrin does not have mutagenic activity to any strain with or without metabolic activation.

The potential of Deltamethrin to induce gene mutations in mammalian cells was also checked in 2016 in an HPRT test on Chinese hamster V79 cell line ([redacted]; 2017; M-577646-01-1). The treatment period was 4 hours with and without metabolic activation. In the main experiment precipitation was observed at 1000.0 µg/mL in the absence of metabolic activation and at 500.0 µg/mL and above in the presence of metabolic activation. No relevant cytotoxic effects occurred up to the maximum concentration with and without metabolic activation. No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed up to the maximum concentration. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental parts. Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system. Deltamethrin did not induce gene mutations at the HPRT locus in V79 cells and was therefore considered to be non-mutagenic in this HPRT assay.

Deltamethrin potential to induce micronuclei in human lymphocytes was assessed in an in vitro micronucleus test ([redacted]; 2017; M-577648-01-1) after 4 hours or 20 hours exposure without S9 mix and after 4 hours exposure with S9mix. In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation. In the absence and presence of S9 mix, no relevant increase in the number of micronucleated cells was observed after treatment with the test item. Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with micronuclei. Deltamethrin was considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to precipitating concentrations.

Based on the full genotoxicity package including *in vitro* and *in vivo* studies performed on deltamethrin and the evaluation of the toxicological potential of all specified or potential impurities (see document on the toxicological assessment of the technical specification, M-480329-05-1), it can be concluded that deltamethrin is non genotoxic compound.

Photomutagenicity

According to the new data requirements (Commission regulation (EU) N° 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013), the conduct of a photomutagenicity study should be considered if the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is greater than 1000 L x mol^{-1} x cm^{-1}, and if the structure of the molecule indicates a potential for photomutagenicity.

For deltamethrin there is no evidence of a photoreactivity potential (see chapter CA 5.2.7; KCA 5.2.7/01, M-466174-01-1) and the Ultraviolet/visible molar extinction/absorption coefficient is smaller than 1000 L x mol^{-1} x cm^{-1}. As concluded by [redacted]. in 2011 (M-465377-01-1), if an *in vitro* 3T3 NRU phototoxicity test is negative there is no need for a photogenotoxicity study. Given the similarity of the underlying principles involved in inducing the different endpoints it is very unlikely that a clearly non-phototoxic compound could have a relevant photogenotoxic potential.

Table 5.4-01: Summary on genotoxicity studies

Test system Study reference	Test object	Concentration	Result
Ames test [redacted] 1980, M-175920-01-1	S.typhimurium TA98, TA100, TA1535, TA1537, TA1538	2 - 5000 µg/plate	negative
	E. Coli WP2 uvrA/pKM101	1250 – 5000 µg/plate	negative
Ames test [redacted] 1984, M-124957-01-1	S.typhimurium TA98, TA100	20 – 600 µg/plate	negative

Test system Study reference	Test object	Concentration	Result
Ames test [redacted]**2005,** M-253266-01-2	S.typhimurium TA98, TA100, TA1535, TA1537	5 – 5000 µg/plate	negative
	E. Coli WP2 uvrA/pKM101	5 – 5000 µg/plate	negative
Gene mutation test [redacted]1984, M-124957-01-1	Chinese hamster lung cells (V79)	4-40 µg/ml	negative
Chromosome aberration [redacted], 1989, M-175946-01-1	Chinese hamster ovary cells	9.5-150 µg/mL	negative
Micronucleus test [redacted], 1983, M-124931-01-1	mice (males & females)	16 mg/kg oral in corn oil	negative
Unscheduled DNA synthesis test [redacted]1989, M-149338-01-1	rat primary hepatocytes*	42-4200 µg/mL	negative
Dominant lethal assay (germ cells) [redacted] & [redacted], 1977, M-149340-01-2	Mice (males only)	6 & 15 mg/kg single dose, 3 mg/kg/day for 7 days	negative
In vitro MNT, [redacted]**2017** M-577648-01-1	Human lymphocytes	5.4 – 50.8 µg/mL, 4 hours +/- S9, 20 hours –S9	negative
HPRT test [redacted] **2017** M-577646-01-1	Chinese hamster V79 cells	31.3 – 1000 µg/mL	negative
In vivo Cytogenetic test [redacted] 1983, M-124958-01-1	Mice (females only)	1.36, 3.4, 6.8 mg/kg in olive oil single dose or for 5 days	negative

Comparison with classifiaction criteria:

There was no indication that deltamethrin has a mutagenic effect on somatic or germ cells in several in vitro and in vivo assays. The criteria for classification for mutagenicity were not met.

Conclusion on classification and labelling:

CLP Regulation: No classification

CA 5.4.1 *In vitro* studies

In addition to the *in vitro* studies already available in the Monograph and baseline dossier, a new Ames test study was conducted in 2005 at the request of Japanese authorities.

Report:	KCA 5.4.1/01; [redacted]; 1980; M-175920-01-1
Title:	Detection of a mutagenic potency of Decamethrin (RU 22974). Bacterial tests
Report No.:	A98112
Document No.:	M-175920-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Growth inhibition test

Deltamethrin (purity 99%) was evaluated for mutagenic activity according to a growth inhibition test using the Slater diffusion method. The method is based upon the observation that E, Coli mutants, either DNA polymerase deficient, or carrying a mutation responsible for an increased X-ray or U.V. sensitivity, are more sensitive than the mother strains to the bactericidal action of DNA-altering compounds. Dose levels were 1250, 2500 and 5000 µg deltamethrin/ml. The solvent used was dimethyl sulfoxid (DMSO). Positive control was N-methyl N'-nitro-N-nitrosoguanidine (MNNG).

No guideline available.

Ames test

Deltamethrin (purity 99%), was evaluated for mutagenic activity in the Ames test using *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 with and without metabolic activation (rat liver S9-mix). The dose levels were 0, 2, 10, 50, 200, 500, 1000 and 5000 µg/plate. The solvent used was dimethyl sulfoxide (DMSO). Positive controls were N-methyl N'-nitro-N-nitrosoguanidine (MNNG), aminoacridine, nitrofluorene and 2-aminoanthracen.

The study follows the OECD guideline 471 revised in July 1997, except that *Salmonella typhimurium* strain TA102 or *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101) were not used. Only one negative control was used (the untreated control).

Results

Deltamethrin did not exhibit any mutagenic activity in the "growth inhibition test". In contrast to control mutagens, decamethon had the same effect on *E. Coli* mother strains and their mutants. Deltamethrin was neither found to be mutagenic in *Salmonella typhimurium* strains TA 1535, TA 100, TA 1537, TA 1538 and TA 98 with and without metabolic activation.

Comments from the former RMS Sweden

No signs of mutagenic potency of deltamethrin could be detected in the bacterial test systems used. No OECD guidelines exist for the first type of study. The second one follows OECD guideline no 471. There are no statements concerning GLP or Quality Assurance inspections but both studies seem to be of acceptable quality.

Report:	KCA 5.4.1/03; [redacted] 1984; M-124957-01-1
Title:	Lack of mutagenicity of synthetic pyrethroids in Salmonella typhimurium strains and in V79 Chinese hamster cells
Report No.:	A41894
Document No.:	M-124957-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Mutagenicity assays with *Salmonella typhimurium*

Deltamethrin (purity *96%*) and other pyrethroids (cypermethrin, permethrin, bioresmethrin, resmethrin, cismethrin and fenvalerate) were evaluated for mutagenic activity in *Salmonella typhimurium* strains TA 100 and TA 98 with and without metabolic activation (Aroclor induced rat liver S9-mix) using the plate incorporation assay and microscale-fluctuation test. The dose levels were 0, 20, 60, 200 and 600 µg of deltamethrin per plate in the plate incorporation assay and 0, 1, 4 and 40 µg deltamethrin/ml in the fluctuation test. The solvent used was dimethyl sulfoxid (DMSO). Positive controls were methyl methanesulphonate (MMS); benzopyrene (BP) and 4-nitroquinoline N-oxide (NQO).

Mutagenicity assays with V79 Chinese hamster cells

Deltamethrin (purity 96%) and other pyrethroids (cypermethrin, permethrin, bioresmethrin, resmethrin, cismethrin and fenvalerate) were evaluated for mutagenic activity in the HPRT-locus and Na^+/K^+ ATPase-locus) in V79 Chinese hamster cells in the presence and absence of primary rat hepatocytes. The dose levels were 0,2, 20 and 40 µg deltamethrin/ml. Positive controls were N-methyl-N-nitrosurea (MNU) and N-nitrosodimethylamine (NDMA).

Results

Deltamethrin was not found to be mutagenic in *Salmonella typhimurium* strains TA 100 or TA 98 in the presence or absence of rat liver activation system using the plate incorporation assay or the microscale-fluctuation test. Deltamethrin was not found to be mutagenic for either genetic locus (HPRT-locus, NA^+/K^+ ATPase-locus) in V79 cells when tested in either the presence or the absence of rat hepatocytes. Deltamethrin was not toxic in presence or absence of rat hepatocytes tested at concentrations up to 40 ng/ml in V79 Chinese hamster cells. No cytotoxicity was observed.

Comments from the former RMS Sweden

The reference is a published article (Mutation Research 137 (1984) 7-15). There are some deviations from OECD guidelines no 471 and 476. Only two strains of Salmonella typhimurium were used in the plate incorporation assay and the fluctuation test. Only one negative control was used (the untreated control). According to the guidelines no 471 and 476, both untreated and solvent controls should be included in each experiment. Under the test condition used in this study, deltamethrin was not found to be mutagenic in the Ames test. A serious shortcoming concerning the mutagenicity assays with V79 Chinese hamster cells is that no cytotoxicity was observed which indicates that the dose levels were too low. The medium in which the test substance was dissolved in, was not specified. There is no information concerning GLP standards or Quality Assurance inspections. The mutagenicity assays with V79 Chinese hamster cells is not of acceptable quality.

Report:	KCA 5.4.1/04; [redacted]; 1989; M-175946-01-1
Title:	Chromosome aberration assay of deltamethrin in Chinese hamster ovary
Report No.:	A98126
Document No.:	M-175946-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Deltamethrin (purity 99.2%) dissolved in acetone, was tested in the chromosome aberration assay using Chinese hamster ovary cells with or without metabolic activation (Aroclor-induced rat liver S9-mix) at dose levels of 0, 9.5, 19, 38, 75 and 150 µg/ml. The test article was tested to its limit of solubility in culture medium in both the non-activated and S-9 activated test systems. Metaphase cells were collected at 18 h after treatment over the entire cell cycle in the non-activated system and at 8 and 12 h following a 2 h treatment in the S9-activated system. Positive controls were triethylenemelanine (TEM) and cyclophosphamide (CP). Negative controls were acetone and untreated cells (growth medium).

This study was performed under GLP conditions. All technical-related requirements of OECD guideline 473 are fulfilled. The test article was tested to its limit of solubility in culture medium in both the nonactivated and S-9 activated test systems. The required number of 300 metaphases was fulfilled. The positive controls gave the expected results and thus confirmed the sensitivity of the study method. The study is fully valid for this endpoint. Deltamethrin was concluded to be negative in the CHO cytogenetics assay.

Results

At the time of harvest, dose levels 38, 75 and 150 µg/ml were observed to be slightly toxic upon microscopic examination of the cell monolayer when tested without metabolic activation. Dose level 150 µg/ml at 12 hours was observed to be slightly toxic upon microscopic examination of the cell monolayer when tested with metabolic activation. No significant increase in chromosome aberrations was observed in the non-activated or S9 activated test system at any harvest time.

Comments from the former RMS Sweden

Under the conditions of the assay described in this study, deltamethrin was concluded to be negative in the CHO cytogenetic assay. The study follows OECD guideline no 473. It was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections, and seems to be of acceptable quality.

Report:	KCA 5.4.2/02; [redacted]; 1989; M-149338-01-1
Title:	Unscheduled DNA synthesis of deltamethrin in rat primary hepatocytes.
Report No.:	A70853
Document No.:	M-149338-01-1
Guideline(s):	USEPA (=EPA): 84-2
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 92%) was dissolved in acetone and tested for its potential to induce DNA damage in Fischer 344 adult male rat hepatocytes *in vitro*. The hepatocytes were cultured for 18-20 h in the presence of deltamethrin at concentrations of 42, 130, 420, 1300 and 4200 µg/ml together with ^{3}H-thymidine. 7, 12-dimethylbenzanthracene (7,12-DMBA) dissolved in dimethyl sulfoxide (DMSO) was used as positive control. Acetone was used as the solvent control for the test article.

This study was performed under GLP conditions. All substance- and culture-related requirements of OECD guideline 482 are fulfilled, the highest concentration chosen for the UDS assay was at the limit of solubility of the test article in the solvent. A positive control gave the expected results and thus confirmed the sensitivity of the study method. The study is fully valid for this endpoint.

Results

Deltamethrin was not cytotoxic at any dose level. No increases in net nuclear grains per cell were seen at concentrations of 42, 130, 420, 1300 and 4200 µg/ml compared to the appropriate solvent control. The positive control (7,12-DMBA) caused significant increases in the mean number of net nuclear grain counts over that in the solvent control.

Comments from the former RMS Sweden

When examined at concentrations up to 4200 µg/ml (the limit of solubility of the test substance in the solvent), deltamethrin did not induce unscheduled DNA synthesis in primary cultures of rat hepatocytes under the test conditions used in this study. The study follows OECD guideline no 482. It was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality

Report:	KCA 5.4.1/05; [redacted]; 2005; M-253266-01-2
Title:	Deltamethrin: reverse mutation test in bacterial system
Report No.:	NR05215
Document No.:	M-253266-01-2
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive Summary

In this *in vitro* assessment of the mutagenic potential of deltamethrin (Batch 2350014, 98.8 % of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98 and TA 100 and tryptophan dependent mutants of Escherichia coli, strains WP2uvrA/pKM101 were exposed to deltamethrin diluted in dimethyl sulphoxide (DMSO) at concentrations up to 5000 µg/plate. For each bacterial strain and dose level, triplicate plates were used in both the presence and absence of an phenobarbital and 5,6-benzoflavone-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.

Growth inhibition was not observed up to 5000 µg/plate. The precipitation on plates was noted at 5000 µg/plate.
Deltamethrin did not cause any significant increase in the number of revertant colonies in either the presence or absence of metabolic activation.

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, Deltamethrin was non-mutagenic with or without S9 mix in the pre-incubation modification of the Salmonella/microsome test.

I. Materials and Methods

A. Material

1. Test Material:	Deltamethrin
Description:	White powder
Lot/Batch:	2350014
Purity:	98.8%
CAS:	52918-63-5
Stability of test compound:	Stable for 5 hours at room temperature
2. Control materials:	
Negative:	Culture medium
Solvent:	DMSO
Positive:	Sodium azide (Sigma) for TA 1535 at 0.5 µg/plate without S9 mix 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (Wako) for TA98, TA100, WP2uvrA/pKM101 without S9 mix 9-Aminoacridine (Sigma) for TA1537 at 80 µg/plate without S9 mix 2-Aminoanthracene (Wako) at 0.5 µg/plate for TA98, at 1 µg/plate for TA100 at 2 µg/plate for TA1535, TA1537 and WP2uvrA/pKM101 with S9 mix
3. Test organisms:	
Species:	Salmonella typhimurium LT2 mutants
Strain:	Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, and TA 98
Source:	Strains obtained from the [redacted] (Japan)
Sepcies	Escherichia coli
Strain	WP2uvrA/pKM101
Source	Strains obtained from [redacted] (Japan)
4. Test compound concentrations:	
Range-finding	First assay for all strains with or without S9 mix: 5,20,80,313,1250 and 5000 µg/plate
Pre-incubation assay:	For all strains with or without S9 mix: 313, 625, 1250, 2500 and 5000 µg/plate

B. Study Design and methods

The experimental phase of the study was performed between February 22 to March 10, 2005 at [redacted] Japan.

The test is an *in vitro* screening method which detects point mutations caused by chemical agents. Auxotrophic mutants of Salmonella typhimurium or Esherichia coli are used to demonstrate this

effect. For this purpose, the rate of reversion to prototrophy is evaluated in negative control and treated groups.

1. Pre-incubation assay

In the non-metabolic activation system, a 0.5 mL of 0.1 M sodium phosphate buffer solution (pH 7.4) was poured into sterilized test tubes, and each 0.1 mL of bacterial suspensions associated with each 0.1 mL of the test substance solutions or control substance solution was added and mixed. Then, each test tube was pre-incubated for 20 minutes at 37°C. After pre-incubation, each 2 mL of molten top agar with biotin – histidine or tryptophan kept at 45 °C was added to each test tube and then each of the mixture was poured on a minimal glucose agar plate and the plates were solidified at room temperature. These plates were incubated for 48 hours at 37°C.

As for the metabolic activation system, a 0.5 mL of S-9 Mix (10%), instead of a sodium phosphate buffer solution in the non-metabolic activation system, was poured into sterilized tubes and then the same operation in the non-metabolic activation system was conducted.

After incubation, revertant colonies on the plate were counted. Three plates were used in each test solution and control one.

2. Assessment criteria

In order to evaluate the results of a mutation potential of the test substance, the mean number of the revertant colonies of each test system were calculated and compared to that of the solvent control.

If more than two fold and dose-related increase in revertant colonies were observed as compared with the solvent control, it was evaluated as positive in terms of mutagenicity.

II. Results and discussion

There was no indication of a bacteriotoxic effect of deltamethrin at doses of up to 5000 µg per plate. Precipitation on plates was observed at 5000 µg per plate. Precipitation on plates was observed at 5000 µg per plate. The total bacteria counts consistently produced results comparable to the negative controls or differed only insignificantly. In both tests, range finding study or main study, no significant increase in revertant colonies was observed for any test strains under either condition with or without metabolic activation.

In cases of the positive control substances, AF-2, NaN$_3$ and 9-AA without S-9 Mix and 2-AA with S-9 Mix, a marked increase in revertant colonies was observed for each strain.

Therefore, it is concluded that the mutagenic effect of Deltamethrin was negative to all test strains in both conditions with and without metabolic activation for the reverse mutation test in bacterial system.

Table 5.4.1 – 01: Mean mutant values per plate in the range-finding assay

Test item	Concentration µg/plate	S9 mix	Strains				
			TA100	TA1535	TA98	TA1537	WP2uvrA/ pKM101
Deltamethrin	0	-	113	10	17	8	69
	5		111	10	18	5	79
	20		101	10	17	5	71
	80		71	8	16	6	78
	313		113	10	19	8	74
	1250		106	11	19	8	75
	5000		114	11	18	8	83
	0	+	101	13	29	7	110
	5		116	10	34	8	102
	20		114	11	22	9	115
	80		96	12	27	10	105
	313		112	10	25	9	107
	1250		117	10	20		102
	5000		107	10	21	11	110
AF-2	0.01	-	707				1596
	0.1				396		
NaN_3	0.5			259			
9-AA	80					348	
2-AA	0.5	+			251		
	1		658				
	2			237		178	337

Table 5.4.1-02: Mean mutant values per plate in the pre-incubation assay

Test item	Concentration µg/plate	S9 mix	Strains				
			TA100	TA1535	TA98	TA1537	WP2uvrA/pKM101
Deltamethrin	0	-	119	10	19	7	65
	313		116	8	18	7	76
	625		113	7	18	5	66
	1250		122	8	21	8	66
	2500		115	10	8	7	67
	5000		110	11	20	6	67
	0	+	114	10	26	8	118
	313		112	7	28	7	110
	625		105	10	20	6	102
	1250		106	8	25	8	108
	2500		112	9	20	8	104
	5000		109	8	20	8	106
AF-2	0.01	-	898				1591
	0.1				378		
NaN_3	0.5			251			
9-AA	80					482	
2-AA	0.5	+			258		
	1		712				
	2			271		169	378

III. Conclusions

The mutagenic activity of test substance deltamethrin was evaluated to be negative for the reverse mutation test in bacterial system.

Report: KCA 5.4.1/07; [redacted]; 2017; M-577646-01-1
Title: Deltamethrin (AE F032640): Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT)
Report No.: 1805901
Document No.: M-577646-01-1
Guideline(s): OECD Guidelines for the Testing of Chemicals No. 476 in Vitro Mammalian Cell Gene Mutation Tests using the Hprt and xprt genes“ (adopted 29 July 2016)
Commission Regulation (EC) No. 440/2008 B.17: Mutagenicity In vitro Mammalian Cell Gene Mutation Test, dated May 30, 2008.
United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5300, In vitro Mammalian Cell Gene Mutation Test, EPA 712-C-98-221, August 1998.
Japanese Guidelines: Kanpoan No. 287 -- Environment Protection Agency Eisei No. 127 -- Ministry of Health & Welfare Heisei 09/10/31 Kikyoku No. 2 -- Ministry of International Trade & Industry
Guideline deviation(s): none
GLP/GEP: **yes**

Executive Summary

The study was performed to investigate the potential of Deltamethrin (AE F032640) to induce gene mutations at the HPRT locus in V79 cells of the Chinese hamster.
The treatment period was 4 hours with and without metabolic activation.
The highest applied concentration in the pre-test on toxicity was 2000 µg/mL with respect to the current OECD Guideline 476. The concentration range of the main experiment was limited by precipitation of the test item.
In the main experiment precipitation was observed at 1000.0 µg/mL in the absence of metabolic activation and at 500.0 µg/mL and above in the presence of metabolic activation.

No relevant cytotoxic effects occurred up to the maximum concentration with and without metabolic activation.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed up to the maximum concentration.

No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental parts.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system.

Conclusion:

In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells.

Therefore, Deltamethrin (AE F032640) is considered to be non-mutagenic in this HPRT assay.

I. Materials and Methods

A. Material

1. Test Material:	Deltamethrin (AE F032640)
Description:	White solid
Lot/Batch:	PMHDN001265
Purity:	99.6 % w/w (HPLC)
CAS:	52918-63-5
Stability of test compound:	Formulations freshly prepared and use within two hours of preparation. Solutions kept at room temperature.

2. Control materials:	
Negative:	Culture medium: MEM (minimal essential medium) containing Hank's salts, neomycin (5 µg/mL), 10% FBS, and amphotericin B (1 %). During treatment no FBS was added to the medium. For the selection of mutant cells the complete medium was supplemented with 11 µg/mL 6-thioguanine. All cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO2 (98.5 % air).

	PBS solution: pH 7.2, containing 8000 mg/L NaCl, 200 mg/L KCl, 150 mg/L Na_2HPO_4 and 200 mg/L KH_2PO_4 "Saline G" solution: pH 7.2, containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4
Solvent:	0.5 % THF (tetrahydrofuran); Purity: 99.85 %
Positive:	Without metabolic activation: EMS; ethylmethane sulfonate; Purity: 99 %; Dissolved in nutrient medium; Concentration: 300 µg/mL = 2.4 mM With metabolic activation: DMBA; 7,12-dimethylbenz(a)anthracene; Purity: ≥ 95 %; Dissolved in DMSO (final concentration in nutrient medium 0.5 %); Concentration: 2.3 µg/mL = 8.9 µM

3. Test organisms:

Species:	V79 cell line
Strain:	Before freezing, the level of spontaneous mutants may be reduced by treatment with HAT-medium. Each master cell stock is screened for mycoplasm contamination and checked for karyotype stability and spontaneous mutant frequency. The cultures were incubated at 37 °C in a humidified atmosphere with 1.5 % CO2.
Source:	Supplied by [redacted] Germany
S9 preparation	Phenobarbital/β-naphthoflavone induced rat liver S9 Protein concentration of the S9 preparation used: 28.1 mg/mL Preparation and storage according to the current version of Envigo SOP for rat liver S9 preparation

4. Test compound concentrations:

Pre-experiment	With or without S9 mix: 15.6, 31.3, 62.5, 125.0, 250.0, 500.0, 1000.0, 2000.0 µg/mL
Main experiment	With or without S9 mix: 31.3, 62.5, 125.0, 250.0, 500.0, 1000.0 µg/mL

B. Study Design and methods

Dose Selection and pre-test:

Dose selection was performed according to the OECD Guideline for Cell Gene Mutation Tests.

The pre-experiment was performed in the presence and absence (4 h treatment) of metabolic activation. Test item concentrations between 15.6 µg/mL and 2000 µg/mL were used. The test medium was checked for precipitation or phase separation at the beginning and at the end of treatment (4 hours) prior to removal to the test item.

The dose range of the main experiment was set according to solubility data generated in the pre-experiment. The cultures at the lowest concentration with and without metabolic activation were not continued as a minimum of only four analysable concentrations is required.

Experimental Performance

Two to four days after sub-cultivation stock cultures were trypsinised and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2% in saline.

After 24 hours the medium was replaced with serum-free medium containing the test item either without S9 mix or with 50 µl/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 hours this medium was replaced with complete medium following two washing steps with "saline G".

Immediately after the end of treatment the cells were trypsinised and sub-cultivated. At least $2.0x10^6$ cells per experimental point (concentration series plus controls) were subcultured in 175 cm² flasks containing 30 mL medium.

Two additional 25 cm² flasks were seeded per experimental point with approx. 500 cells each to determine the relative survival (cloning efficiency I) as measure of test item induced cytotoxicity.

The colonies used to determine the cloning efficiency I were fixed and stained 6 to 8 days after treatment as described below.

Three or four days after first sub-cultivation approximately $2.0x10^6$ cells per experimental point were sub-cultivated in 175 cm² flasks containing 30 mL medium.

Following the expression time of 7 days five 75 cm² cell culture flasks were seeded with about 4 to $5x10^5$ cells each in medium containing 6-TG (6-thioguanine). Two additional 25 cm² flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability.

The cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO2 for about 8 days. The colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted.

Statistical analysis

A linear regression was performed to assess a possible dose dependent increase of mutant frequencies. The numbers of mutant colonies generated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05. A t-test was performed using a validated test script of “R to evaluate an isolated increase of the mutation frequency at a test point exceeding the 95% confidence interval. A t-test is judged as significant if the

p-value (probability value) is below 0.05. However, both, biological and statistical significance were considered together.

II. Results and discussion

In the pre-experiment no relevant cytotoxic effects, indicated by a relative cloning efficiency of 50% or below were observed up to the maximum concentration with and without metabolic activation. At the beginning of treatment precipitation was observed at 62.5 µg/ml and above with and without metabolic activation. At the end of the 4 hours treatment precipitation occurred at 500 µg/mL and above with and without metabolic activation. There was no relevant shift of pH and osmolarity of the medium even at the maximum concentration of the test item. The dose range of the main experiment was set according to solubility data generated.

In the main experiment precipitation was observed at 1000.0 µg/mL in the absence of metabolic activation and at 500.0 µg/mL and above in the presence of metabolic activation. No relevant cytotoxic effects indicated by an adjusted cloning efficiency I below 50% in both cultures occurred up to the maximum concentration with and without metabolic activation.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiment up to the maximum concentration.

The 95% confidence interval was exceeded at 62.5 µg/mL in the first culture with metabolic activation (30.2 *vs.* an upper limit of 28.7 mutant colonies/10^6 cells). This isolated increase was judged as irrelevant as it was not reproduced. A t-test evaluating the data of both parallel cultures at this test point showed no significant increase versus the corresponding solvent controls.

A linear regression analysis was performed to assess a possible dose dependent increase of mutant frequencies. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental parts.

In the main experiment with and without S9 mix the range of the solvent controls was from 20.8 up to 29.8 mutants per 10^6 cells; the range of the groups treated with the test item was from 10.9 up to 30.2 mutants per 10^6 cells. The highest solvent control of 29.8 colonies per 10^6 cells slightly exceeded the 95% confidence interval (0.6 – 28.7 colonies per 10^6 cells) but the mean value of both parallel cultures (21.6 and 29.8, equal to a mean of 25.8 colonies per 10^6 cells) remained well within the acceptable range.

EMS (300 µg/mL) and DMBA (2.3 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table 5.4.1 – 05: Summary of results – Main Experiment

		Culture I	Culture II

Main experiment - 4 h treatment	**Concentration (µg/mL)**	**Relative adj. cloning efficiency I (%)**	**Mutant colonies/ 10^6 cells**	**Relative adj. cloning efficiency I (%)**	**Mutant colonies/ 10^6 cells**
Without S9 mix					
Solvent control (THF)	-	100.0	20.8	100.0	24.7
Positive control (EMS)	300.0	76.1	282.1	101.2	235.0
Deltamethrin	31.3	76.9	#	55.4	#
	62.5	65.8	29.3	62.1	26.5
	125.0	85.5	19.3	70.1	12.7
	250.0	91.4	13.6	81.1	13.8
	500.0	99.6	21.2	89.3	27.0
	1000.0 P	83.1	24.9	88.5	21.6
With S9 mix					
Solvent control (THF)	-	100.0	21.6	100.0	29.8
Positive control (DMBA)	2.3	62.8	212.3	96.9	249.5
Deltamethrin	31.3	87.9	#	84.8	#
	62.5	88.2	30.2	92.3	14.6
	125.0	79.7	19.4	100.2	18.2
	250.0	70.0	23.1	83.2	22.0
	500.0 P	78.0	16.8	100.5	23.5
	1000.0 P	69.4	17.3	98.8	10.9

P precipitation visible at the end of treatment; Adj.: adjusted
culture was not continued as a minimum of only four analysable concentrations is required
95% confidence interval, without S9 mix: 0.2-29.7; with S9 mix: 0.6-28.7 mutant colonies/10^6 cells

III. Conclusions

In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, Deltamethrin (AE F032640) is considered to be non-mutagenic in this HPRT assay.

Report:	KCA 5.4/06; [redacted]; 2017; M-577648-01-1
Title:	Deltamethrin (AE F032640): Micronucleus test in human lymphocytes in vitro
Report No.:	1805902
Document No.:	M-577648-01-1
Guideline(s):	- OECD Guideline for the Testing of Chemicals No. 487 In vitro Mammalian Cell Micronucleus Test, adopted 29 July 2016 - EC Commission Directive 2004/10/EC
Guideline deviation(s):	none
GLP/GEP:	yes

Executive Summary

The test item deltamethrin (AE F032640), dissolved in tetrahydrofuran (THF), was assessed for its potential to induce micronuclei in human lymphocytes *in vitro* in two independent experiments. The following study design was performed:

	Without S9 mix		With S9 mix
	Exp. I	Exp. II	Exp. I
Stimulation period	48 hrs	48 hrs	48 hrs
Exposure period	4 hrs	20 hrs	4 hrs
Recovery	16 hrs	—	16 hrs
Cytochalasin B exposure	20 hrs	20 hrs	20 hrs
Total culture period	88 hrs	88 hrs	88 hrs

In each experimental group two parallel cultures were analyzed. 1000 binucleated cells per culture were evaluated for cytogenetic damage. To determine a cytotoxic effect the Cytokinesis-block proliferation index (CBPI) was determined in 500 cells per culture and cytotoxicity is described as % cytostatis.

The highest applied concentration in this study (2000 µg/mL of the test item) was chosen with respect to the current OECD Guideline 487.

Precipitation of the test item in the culture medium was observed in Experiment I at 16.6 µg/mL and above in the absence of S9 mix and at 50.8 µg/mL and above in the presence of S9 mix and in Experiment II at 26.3 µg/mL and above in the absence of S9 mix at the end of treatment. No relevant influence on osmolarity or pH was observed.

In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation.

In the absence and presence of S9 mix, no relevant increase in the number of micronucleated cells was observed after treatment with the test item.

Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with micronuclei.

Conclusion:

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes.

Therefore, Deltamethrin (AE F032640) is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to precipitating concentrations.

I. Materials and Methods

A. Material

1. Test Material: Deltamethrin (AE F032640)

Description: White solid

Lot/Batch:	PMDN001265
Purity:	99.6 % w/w (HPLC)
CAS:	52918-63-5
Stability of test compound:	Formulations freshly prepared and use within two hours of preparation. Solutions kept at room temperature.

2. Control materials:

Negative:	Culture medium: DMEM/F12 (1:1) including 200 mM GlutaMAX™ and supplemented with penicillin/streptomycin (100 U/mL/100 µg/mL), the mitogen PHA (3 µg/mL), 10 % FBS (fetal bovine serum), 10 mM HEPES and the anticoagulant heparin (125 U/S.P.-U/mL)
	"Saline G" solution: pH 7.2, containing 8000 mg/L NaCl, 400 mg/L KCl, 1100 mg/L glucose • H_2O, 192 mg/L Na_2HPO_4 • 2 H_2O and 150 mg/L KH_2PO_4
Solvent:	0.5 % THF (tetrahydrofuran); Purity: 99.85 %
Positive:	Without metabolic activation:
	Name: MMC mitomycin C (pulse treatment) Purity: 98 % Dissolved in: Deionized water Concentration: 1.0 µg/mL
	Name: Demecolcin (continuous treatment) Purity: ~ 98 % Dissolved in: Deionized water Concentration: 75 ng/mL
	With metabolic activation:
	Name: CPA cyclophosphamide Purity: 97.0 – 103.0 % Dissolved in: Saline (0.9 % NaCl [w/v]) Concentration: 17.5 µg/mL

3. Test organisms:

Species:	Human blood cultures
Strain:	Blood collected from two healthy non-smoking male donors; 22 and 24 years old for Experiment I and II, respectively.
Source:	Human lymphocytes were stimulated for proliferation with the mitogen PHA to culture medium for a period of 48 hours prior treatment.
	The lymphocytes of the respective donors have been shown to respond well to stimulation of proliferation with PHA and to positive control substances.

S9 preparation	Phenobarbital/β-naphthoflavone induced rat liver S9
	Protein concentration of the S9 preparation used: 28.1 mg/mL
	Preparation and storage according to the current version of Envigo SOP for rat liver S9 preparation

4. Test compound concentrations:

Experiment I (pre-experiment)	With or without S9 mix (preparation interval 40 hours; exposure period: 4 hours): 1.8, 3.1, 5.4, 9.5, 16.6, 29.0, 50.8, 88.9, 222.0, 667.0, 2000.0 µg/mL
Experiment II	Without S9 mix (preparation interval 40 hours; exposure period: 20 hours): 5.2, 7.8, 11.7, 17.6, 26.3, 39.5, 59.3, 88.9, 133.0, 200.0 µg/mL

B. Study Design and methods

Dose Selection and pre-test:

Dose selection was performed according to the current OECD Guideline for the *in vitro* micronucleus test.

2000 µg/mL were applied as top concentration for treatment of the cultures in the pre-test. Test item concentrations ranging from 1.8 to 2000 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 16.6 µg/mL and above in the absence of S9 mix and at 50.8 µg/mL and above in the presence of S9 mix. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Using a reduced Cytokinesis-block proliferation index (CBPI) as an indicator for toxicity, no cytotoxic effects were observed in Experiment I after 4 hours treatment in the absence and presence of S9 mix. Considering the precipitation data of Experiment I, 200 µg/mL was chosen as top treatment concentration for Experiment II.

Cytogenetic Experiment:

- For pulse exposure (**Experiment I**): About 48 hrs after seeding two blood cultures were set up in parallel for each test item concentration. The culture medium was replaced with serum-free medium containing the test item. For the treatment with metabolic activation S9 mix was added to culture medium (final protein concentration: 28.1 mg/mL). After 4 hrs the cells were spun down by gentle centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with "saline G". The washing procedure was repeated once. The cells were resuspended in complete culture medium with 10 % FBS (v/v) and cultured for a 16-hour recovery period. After this period Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.
- For continuous exposure (without S9 mix; **Experiment II**): About 48 hrs after seeding two blood cultures were set up in parallel for each test item concentration. The culture medium was replaced with complete medium containing the test item. After 20 hours the cells were spun down by gentle

centrifugation for 5 minutes. The supernatant was discarded and the cells were resuspended in and washed with "saline G". The washing procedure was repeated once. The cells were resuspended in complete culture medium with 10 % FBS (v/v) and Cytochalasin B (4 µg/mL) was added and the cells were cultured another approximately 20 hours until preparation.

Preparation of cells

The cultures were harvested by centrifugation 40 hrs after beginning of treatment. After washing operations, the cells were resuspended in 5 mL KCl solution (0.0375 M) and incubated at 37 °C for 20 minutes. 1 mL of ice-cold fixative mixture of methanol and glacial acetic acid (ratio 19:1) was added to the hypotonic solution and the cells were resuspended carefully. After removal of the solution by centrifugation the cells were resuspended for 2 x 20 minutes in fixative and kept cold. The slides were prepared by dropping the cell suspension in fresh fixative onto a clean microscope slide. The cells were stained with Giemsa.

Evaluation of cytotoxicity and cytogenetic damage

Evaluation of the slides was performed using microscopes with 40 x objectives. The micronuclei were counted in cells showing a clearly visible cytoplasm area. The criteria for the evaluation of micronuclei are described in the publication of Countryman and Heddle (1976). The micronuclei have to be stained in the same way as the main nucleus. The area of the micronucleus should not extend the third part of the area of the main nucleus. 1000 binucleate cells per culture were scored for cytogenetic damage on coded slides. The frequency of micronucleated cells was reported as % micronucleated cells. To describe a cytotoxic effect the CBPI was determined in 500 cells per culture and cytotoxicity is expressed as % cytostasis. A CBPI of 1 (all cells are mononucleate) is equivalent to 100 % cytostasis.

$$CBPI = \frac{(MONC \ x\ 1) + (BINC \ x\ 2) + (MUNC \ x\ 3)}{n}$$

CBPI Cytokinesis-block proliferation index
n Total number of cells
MONC Mononucleate cells
BINC Binucleate cells
MUNC Multinucleate cells

$$Cytostasis\ \% = 100 - 100\ [(CBPIT - 1) / (CBPIC - 1)]$$

T Test item
C Solvent control

Statistical analysis

Statistical significance was confirmed by the Chi square test (α < 0.05), using a validated test script of "*R*". Within this test script a statistical analysis was conducted for those values that indicated an increase in the number of cells with micronuclei compared to the concurrent solvent control.

II. Results and discussion

Precipitation of the test item in the culture medium was observed in Experiment I at 16.6 µg/mL and above in the absence of S9 mix and at 50.8 µg/mL and above in the presence of S9 mix and in Experiment II at 26.3 µg/mL and above in the absence of S9 mix at the end of treatment. No relevant influence on osmolarity or pH was observed.

Table 5.4.1 – 03: Tested concentrations and evaluated experimental points

Exp.	Prep. interval	Exposure period	Concentrations in µg/mL									
Without S9 mix												
I	**40 hrs**	**4 hrs**	**1.8**	**3.1**	**5.4**	**9.5**	**16.6[P]**	**29.0**	**50.8[P]**	**88.9[P]**	**222[P]**	**667[P]**	**2000[P]**
II	**40 hrs**	**20 hrs**		**5.2**	**7.8**	11.7	17.6	**26.3[P]**	**39.5[P]**	**59.3[P]**	**88.9[P]**	**133[P]**	**200[P]**
With S9 mix												
I	**40 hrs**	**4 hrs**	**1.8**	**3.1**	**5.4**	**9.5**	16.6	29.0	50.8[P]	**88.9[P]**	**222[P]**	**667[P]**	**2000[P]**

[P] Evaluated experimental points are shown in bold characters
Precipitation was observed at the end of treatment

In the absence and presence of S9 mix, no cytotoxicity was observed up to the highest evaluated concentration, which showed precipitation.
In the absence and presence of S9 mix, no relevant increase in the number of micronucleated cells was observed after treatment with the test item.
In both experiments, either Demecolcin (75 ng/mL), MMC (1.0 µg/mL) or CPA (17.5 µg/mL) were used as positive controls and showed distinct increases in cells with micronuclei.

Table 5.4.1 – 04: Summary of results

Exp.	Preparation interval	Test item concentration in µg/mL	Proliferation index CBPI	Cytostasis in %*	Micronucleated cells in %**
Exposure period 4 hrs without S9 mix					
I	**40 hrs**	Solvent control[1]	**1.85**		**0.85**
		Positive control[2]	**1.78**	**8.7**	10.60[S]
		5.4	**1.93**	**n.c.**	**0.70**
		9.5	**1.82**	**4.3**	**0.65**
		16.6[P]	**1.83**	**3.2**	**0.40**

Exposure period 20 hrs without S9 mix					
II	40 hrs	Solvent control[1]	**1.93**		**0.50**
		Positive control[3]	**1.87**	**6.0**	4.10[S]
		11.7	**1.84**	**9.9**	**0.25**
		17.6	**1.89**	**4.3**	**0.40**
		26.3[P]	**1.88**	**5.8**	**0.15**
Exposure period 4 hrs with S9 mix					
I	40 hrs	Solvent control[1]	**1.92**		**0.75**
		Positive control[4]	**1.71**	**22.6**	2.70[S]
		16.6	**1.95**	**n.c.**	**0.55**
		29.0	**1.96**	**n.c.**	**0.35**
		50.8[P]	**1.88**	**4.7**	**0.25**

* For the positive control groups and the test item treatment groups the values are related to the solvent controls
** The number of micronucleated cells was determined in a sample of 2000 binucleated cells
P Precipitation occurred at the end of treatment
S The number of micronucleated cells is statistically significantly higher than corresponding control values
n.c. Not calculated as the CBPI is equal or higher than the solvent control value
[1] THF 0.5 % (v/v)
[2] MMC 1.0 µg/mL
[3] Demecolcin 75 ng/mL
[4] CPA 17.5 µg/mL

III. Conclusions

In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce micronuclei as determined by the *in vitro* micronucleus test in human lymphocytes. Therefore, deltamethrin is considered to be non-mutagenic in this *in vitro* micronucleus test, when tested up to precipitating concentrations.

CA 5.4.2 *In vivo* studies in somatic cells

These studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.4.2/01; [redacted]; 1983; M-124931-01-1
Title:	Deltamethrin - Detection of a mutagenic potency. Micronucleus test in the mouse
Report No.:	A41868
Document No.:	M-124931-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity not specified) was dissolved in corn oil and administered to 10-week old mice ([redacted]) at a single oral dose of 16 mg/kg bw. Each group consisted of 5 animals/sex. The animals were killed at 24, 48 or 72 h after dosing and the frequencies of micronuclei in bone marrow polychromatic erythrocytes were determined. Positive controls were triethylenemelanine (TEM) and dimethylbenzanthracene (DMBA). Negative controls were corn oil and dimethyl subnoxid (DMSO).

This study is in agreement with the requirements of the current OECD guideline 474 with regard to animal number, dose setting, use of both sexes, sampling times of 24, 48 and 72 hours after treatment and use of a concurrent positive control. Furthermore, the requirement of a minimum of 500 erythrocytes in bone marrow was fulfilled with actual examination of 1000 per animal. The batch number is given so that it could be possible to find information about the purity of the a.i. A quality assurance statement is given in the report confirming that GLP rules were applied. No evidence of treatment-related effects of deltamethrin was obvious, whereas the 2 positive controls confirmed the sensitivity of the study.

Results

One male animal died within 72 h after treatment with deltamethrin. Acute signs of toxicity occurred in all animals administered deltamethrin within 24 h after treatment (piloerection, hyperactivity and locomotor disorders). No statistically significant increase in frequency of micronuclei was observed at any sample time. The ratio of polychromatic to normochromatic erythrocytes was determined for each animal by counting a total of 1000 erythrocytes per animal. The positive controls showed a significant increase in the number of micronuclei at 24, 48 and 72 h.

Comments from the former MS Sweden

Under the test condition used in this study, deltamethrin did not produce micronuclei in the polychromatic erythrocytes in the mouse. The study follows OECD guideline no 474 with exception of the not specified purity of the test substance. There are no statements concerning GLP but the study was subjected to Quality Assurance inspections and seems to be of acceptable quality.

Report:	KCA 5.4.2/03; [redacted]; 1983; M-124958-01-1
Title:	Evaluation of the mutagenic effects of decamthrin: cytoge- netic analysis of bone mirrow
Report No.:	A41895
Document No.:	M-124958-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Deltamethrin (purity 98%), was dissolved in olive oil and administered per os either in single or repeated doses (5 times with intervals of 24 h between doses) to 8 to 12-week old female Swiss mice. The dose levels were 1.4, 3.4 and 6.8 mg/kg bw (the concentrations used represented in turn 1/25, 1/10 and 1/5 of the LD_{50}). Each group consisted of 5 animals. Doses of 2.5 mg/kg bw of colchicine were applied intraperitoneally 2 h before the mice were killed by cervical dislocation. Twenty-four hrs after the last application of deltamethrin, bone marrow was obtained from the femurs of the mice for analysis of chromosome aberrations. Comment: *The interval of 24 hrs was used since the length of the interval of 24-48 h in an additional experiment did not affect the result*
The positive controls received 40 mg/kg bw of cyclophosphamide orally. The negative controls were given olive oil orally or nothing (intact controls).

This published study mainly fulfils the requirements of the current OECD 475 guideline with regard to study design, dosages and animal numbers. GLP status is not mentioned. Only females were used which is in agreement with OECD 475 which agrees that 'most studies could be performed in either sex'. A concurrent positive control (cyclophosphamide) was included, the number of 250 cells examined and the collection time of 24 hours are in agreement with OECD 475. No chromosomal aberration potential of deltamethrin was seen, whereas the positive control results demonstrated the sensitivity of this study.

Results

No animals died in the course of the experiments. No statistically significant increase in the number of aberrations of the chromosome and chromatid exchange type was observed at any dose level after single or repeated administration of deltamethrin. The use of positive and negative controls gave expected results. After repeated application of deltamethrin at a dose of 1.4 mg/kg bw, a significant increase in the frequency of endomitotic reduplication (ER) was noted ($P<0.001$). Comment: *The author was not able to explain the increased frequency of ER. ER was not included among aberrant cells.*

Comments from the former RMS Sweden

Under the test conditions used in this study deltamethrin did not induce chromosomal aberrations in bone marrow of mouse. The reference is a published article (Mutation Research, 120 (1983) 167-171). The study follows OECD guideline no 475 except for the fact that only female mice were used (5 animals/group). According to the guideline at least five female and five male animals per experimental and control group should be employed. The use of a single sex of animals was not justified in this study. There is no information concerning GLP standards or

Quality Assurance inspections. However, the study was well reported and seems to be of acceptable quality.

CA 5.4.3 *In vivo* studies in germ cells

Report:	KCA 5.4.3/01; [redacted]; 1977; M-149340-01-2
Title:	RU 22974: Mutagenic study - Dominant lethal assay in the male mouse.
Report No.:	A20259
Document No.:	M-149340-01-2
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

RU 22974 (deltamethrin) (purity not specified), dissolved in sesame oil was orally administered to 3-month old male mice ([redacted]). The doses were 3 mg/kg bw for 7 days, 6 or 15 mg/kg bw in a single dose (equivalent respectively to 1/10, 1/5 and 1/2 of the LD_{50}). Each group consisted of 10 animals. The mated females were isolated and replaced by virgin females. This procedure was repeated for 8 weeks. The males were killed at the end of the 8th week and testicles were removed for histo-pathological examination. The mated females were killed on day 14 of gestation for examination of the number of implantations and embryonal development. A mutagenic index was established on the basis of the pre- and postimplantation losses. The positive controls received 10 mg/kg bw of triethylene thiophosphoramide. The negative controls received sesame oil, only.

This study mainly fulfils requirements in OECD guideline 478 with regard to study design, doses, running a concurrent positive control group and required observations. Furthermore, the study duration of 8 weeks covered an entire cycle of spermatogenesis. One deviation can be seen in the number of females per mating which should provide at least 400 implants. In this study the numbers ranged from 107 to 153 which, however, were sufficient to detect dominant lethality changes as was confirmed by the dominant lethal effect of the positive control. This study was not conducted under GLP, but the data are robust to conclude that no treatment-related effect was seen due to deltamethrin.

Results

RU 22974 produced no signs of genotoxic activity in the male mouse under the experimental conditions used in this study. RU 22974 was toxic to the male mice in a dose of 15 mg/kg bw (7 out of 20 males died shortly after treatment). The fertility was not affected at the tested doses. No treatment with RU 22974 had an effect on the rate of pre- or postimplantation losses. Histopathological examination of the testicles of all males showed no structural changes. The use of positive controls gave expected results.

Comments from the former RMS Sweden

Deltamethrin was not genotoxic in the dominant lethal assay in the male mouse under the *test* conditions used in this study. The study follows OECD guideline no 478 with exception of the low number of pregnant females in each group, due to the high mortality rate of the male mice. According to the guideline no 478, the number of males in each group should be sufficient to provide between 30 and 50 pregnant females per mating interval. In this study the males in each group provided 6-18 pregnant females per mating interval only, which restricts the sensitivity of the test.

There are no statements concerning GLP standards or Quality Assurance inspections (GLP was not compulsory at the time when this study was performed). Although, the sensitivity of the study was restricted, the study brings some information about the potency of deltamethrin to produce genotoxic activity in the mouse. The results of the study were therefore taken into consideration in this report.

CA 5.5 Long-term toxicity and carcinogenicity

Long term oral toxicity of deltamethrin was investigated in the mouse (97-week and 2-year carcinogenicity studies) and in the rat (2-year carcinogenicity studies). In both species, the nervous system was the main target organ with observation of different neurotoxic signs but not associated with any histopathological findings in the nervous system. No new studies were performed since the last EU review. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter. Additional information, when necessary, will be put in blue and bold.

In the second rat carcinogenicity study ([redacted]; 1995; M-139996-01-1), treatment with deltamethrin was associated with transient neurological effects (uncoordinated movements of the limbs, abnormal or unsteady gait, splayed limbs) in the early part of the study at dietary levels of 500 and 800 ppm (at a time of higher relative test compound intake in terms of mg/kg/day). The NOAEL was set at 25 ppm based on minor biochemical and haematological changes and increased incidence of ballooned cells in the liver of males.

The mouse is the less sensitive species. However, cutaneous lesions such as scars, sores or scabs were observed at the top dose of 2000 ppm in the carcinogenicity study ([redacted]; 1995; M-149308-01-1). Histopathological examinations revealed higher incidence of skin ulceration and cellulitis at 1000 ppm in males and at 2000 ppm in both sexes. These findings are related to the known properties of the test substance on the pain sensors (paresthesia) which could lead the animals to excessive scratching.

Repeat administration of deltamethrin induced also body weight or body weight gain effects in rats often associated with decreased food consumption. The liver was found as a target organ in the rat carcinogenicity study with increase in the incidence and degree of eosinophilic hepatocytes in males at 500 and 800 ppm and increased incidence of ballooned cells in the liver of males at 125 and 800 ppm. No increased incidence of tumors was seen in any carcinogenicity study in mice or rats.

Table 5.5-01: Summary of long term toxicity studies of deltamethrin (studies in the baseline dossier in gray)

Type of study (Document N°) Dose range	NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
2-year rat carcinogenicity study, [redacted] 1980 M-093417-01-1 0, 2, 20, 50 ppm	>50	>2.1/2.8 In M/F	>50	>2.1/2.8 In M/F	slight lower BW at 50 no tumors

2-year rat carcinogenicity study, [redacted] 1995 M-139996-01-1 0, 25, 125, 500, 800 ppm	25	1.1/1.5 In M/F	125/500 In M/F	5.4/29.5 in M/F	↑Ballooned cells in liver of males at 125 ppm ↓BW gain in males from 500 ppm, in females at 800 ppm Week 1, ↓FC week 1 from 500 ppm Incoordinated movements of limbs or abnormal/ unsteady gait in a few males at 500, most males and a few females at 800 no tumors
2-year mouse carcinogenicity study, [redacted] **1980** **M-093412-01-1** **0, 1, 5, 25, 100 ppm**	100	12/15 in M/F	>100	>12/15 in M/F	no significant treatment-related effects no tumors
97-week mouse carcinogenicity study, [redacted] **1995** **M-149308-01-1** **0, 10, 100, 1000, 2000 ppm**	100/1000 in M/F	15.7/189.3 in M/F	1000/2000	155.4/395.1 In M/F	Paresthesia at 1000 in males (skin ulceration & cellutitis) and at 2000 both sexes (scars, sores, scabs), ↑ante mortem signs (emaciation & dyspnea) at 2000 No tumors

Comparison with classification criteria:

The carcinogenic potential of deltamethrin has been evaluated in four carcinogenicity studies (two studies performed in rats and two studies performed in mice), from 2 to 800 ppm in rats and from 0.1 to 2000 ppm in mice. If the dose levels selected in the first studies for both rats and mice were too low, the dose levels selected in the second studies for rats and mice were high enough to induce significant treatment-related effects. In rats, uncoordinated movements of the limbs characterized by splayed limbs and unsteady gait were observed from 500 ppm (22 mg/kg/day) and histopathological findings with ballooned cells in the liver and eosinophilic hepathocytes were observed from 125 ppm (5 mg/kg/day). In mice clinical signs were observed from 1000 ppm (155 mg/kg/day) in males and from 2000 ppm (395 mg/kg/day) in females. There was no treatment-related effect on survival in both species. No evidence of tumorigenic or carcinogenic potential was noticed at any dose level.

Conclusion on classification and labelling:

CLP Regulation: **No classification**

Report:	KCA 5.5/01; [redacted]; 1995; M-139996-01-1
Title:	Deltamethrin (technical) Potential tumorigenic and toxic effects in prolonged dietary administration to rats
Report No.:	A56161
Document No.:	M-139996-01-1
Guideline(s):	JMAF: ; OECD: 453; USEPA (=EPA): Subdivision F, 83-5
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 98.9%) was administered in the diet to rats of the [redacted] BR strain (70 animals/sex/group) at concentrations of 25, 125, 500 and 800 ppm for a period of 104 weeks. The concentrations corresponded to a dose rate of 1, 5, 22 and 36 mg/kg bw/day for males, and 2, 7, 30, 47 mg/kg bw/day for females. The controls (70 animals/sex) received the diet only. Ten rats/sex/group were killed after 52 weeks of treatment for interim toxicity assessment.

The study still complies with the OECD guideline revised in 2009.

Results

Survival at termination was between 40% and 70% for male rats, and between 42% and 52% for female rats. There was an indication of marginally superior survival amongst males at 500 and 800 ppm, compared to controls.

Uncoordinated movements of limbs, characterised by splayed limbs and unsteady gait were noted for males and females receiving 800 ppm and for males receiving 500 ppm. After 8 weeks of treatment these findings were, on the whole no longer apparent.

Statistically significant decreased group mean body weight gain was noted for males at 500 and 800 ppm in comparison with the controls. After the first week of treatment statistically significant decreased group mean body weight gain was noted for females at 800 ppm. Food intake was significantly reduced in comparison with the controls in a dosage-related manner during the first week of treatment for males and females receiving 500 and 800 ppm.

Lymphocyte counts and white cell counts were statistically significantly lower than control for males and females receiving 800 ppm, and females receiving 125 or 500 ppm at the week 13 investigations. Lower lymphocyte counts were also noted at the week 26 investigation for males treated with 125, 500 or 800 ppm **(but not statistically significant).** A slight but significantly lower haemoglobin concentration was noted in week 26 for males treated with 800 ppm compared with controls. Comment: *Fluctuations in haematological values were only observed in weeks 14 and 26, and not at later investigations.*

Table 5.5-01: Summary on lymphocytes counts (L in $10^3/mm^3$) and total white blood cells (WBC in $10^3/mm^3$)

Groups	W13		W26		W52		W78		W104	
	L	WBC	L	WBC	L	WBC	L	WBC	L	WBC
					Males					
control	12.18	14.8	14.34	17.4	7.96	10.9	11.32	14.5	5.89	8.4
25 ppm	10.43	12.7	14.28	17.1	8.93	11.0	12.19	17.2	6.37	9.2
125 ppm	10.11	12.1	13.13	15.8	8.23	10.6	9.89	13.1	5.19	8.3
500 ppm	10.49	12.6	13.18	15.6	7.66	10.4	10.50	15.3	5.96	8.8

800 ppm	9.45*	11.9*	12.69	15.4	7.81	10.0	11.09	15.7	6.04	8.7
					Females					
control	8.25	10.4	6.62	8.1	5.13	6.7	7.15	10.7	3.53	5.5
25 ppm	8.55	10.1	7.81	10.6	5.80	7.9	6.87	9.1	3.58	5.2
125 ppm	6.77*	8.4*	7.78	9.5	5.53	7.2	5.93	7.8	4.29	5.8
500 ppm	6.94*	8.7*	7.47	9.3	5.49	6.9	6.04	7.9	3.67	6.0
800 ppm	5.39**	7.0**	5.68	7.1	5.05	7.1	6.67	8.4	4.07	5.4

*: P<0.05 **: P<0.01

Dosage-related and in some instances statistically significant changes in plasma electrolytes were noted for all treated groups at the week 26, 52, 78 and 104 investigations. **The changes at 25 and 125 ppm were usually observed at only one time point and are therefore not considered significant.**

Table 5.5-02: Mean electrolyte concentrations (mEq/l)

Groups	Na+					Cl-				
	W13	W26	W52	W78	W104	W13	W26	W52	W78	W104
					Males					
control	141	143	144	146	144	100	101	100	101	103
25 ppm	142*	143	143	144	144	102**	101	101*	101	102
125 ppm	143*	143	143	144	145	103**	102	101**	100	104
500 ppm	143**	143	144	146	145	104**	104**	103**	101	104
800 ppm	142**	143	144	145	145	103**	105**	103**	100	104
					Females					
control	140	141	141	139	142	99	99	98	98	98
25 ppm	141	141	140	140	141	101**	101**	100	97	98
125 ppm	141	141	141	140	142	101**	102**	100*	96	101
500 ppm	141	141	141	140	142	103**	103**	101**	97	99
800 ppm	140	141	140	140	142	103**	104**	102**	98	100

Groups	Ca2+					P				
	W13	W26	W52	W78	W104	W13	W26	W52	W78	W104
					Males					
control	5.3	5.5	5.6	5.6	5.5	3.9	3.6	3.3	2.8	3.2
25 ppm	5.4	5.5	5.5	5.7	5.6	3.8	3.5	2.8**	2.7	3.2
125 ppm	5.4	5.4	5.5*	5.6	5.4	3.9	3.3	2.7**	2.6	3.0
500 ppm	5.3	5.5	5.4**	5.5	5.4	3.8	3.4	2.7**	2.5	2.7*
800 ppm	5.3	5.4	5.4**	5.5	5.4	3.9	3.2*	2.7**	2.5*	2.6*
					Females					
control	5.6	5.7	5.8	5.8	5.6	3.6	3.1	2.8	2.3	2.8
25 ppm	5.5	5.6	5.6**	5.5	5.5	3.5	2.9*	2.6	2.3	2.6
125 ppm	5.5	5.5	5.6**	5.5	5.5	3.4	2.8*	2.7	2.4	2.9
500 ppm	5.4*	5.4*	5.6**	5.5	5.5	3.8	2.8**	2.6	2.3	2.8
800 ppm	5.4**	5.4**	5.6**	5.3**	5.5	3.4	2.6**	2.5*	2.2	2.6

*: P<0.05 **: P<0.01

Statistically significant reduction in plasma cholesterol were noted among females at 800 ppm at the week 26 and 52 investigations, and among females and males in all treated groups at the week 78 investigation, **but with no dose-related effects in females and no significant decrease at 25 and 125 ppm in males (see table 5.5-03).** Statistically significant reduced plasma albumin values were noted among females at 500 and 800 ppm on the week 26 and **among females at 800 ppm on the week 78.** A statistically significant decrease in total protein for females treated with 125, 500 or 800 ppm was noted at week 52 and 104, and among females at 800 ppm at week 78 **(see table 5.5-04)**. A statistically significant elevation

in plasma glucose was observed for all treated male groups and for females treated with 800 ppm at the week 52 investigation, and for females treated with 800 ppm at the week 78 and 104 investigations **(see table 5.5-03)**.

Table 5.5-03: Mean plasma glucose (Glc) and cholesterol (CHOL) concentrations (mg/dl)

Groups	W13		W26		W52		W78		W104	
	Glc	CHOL	Glc	CHOL	Glc	CHOL	Glc	CHOL	Glc	CHOL
Males										
control	115	93	145	93	125	112	150	142	118	146
25 ppm	112	79	152	86	149*	105	147	136	128	146
125 ppm	113	87	155	92	146*	114	130	136	130	126
500 ppm	116	82	146	92	142*	94	137	114	136	122
800 ppm	117	86	145	94	149**	108	152	115	121	131
Females										
control	102	110	135	128	121	153	121	144	112	158
25 ppm	103	95	134	109	131	140	132	128	127	130
125 ppm	103	99	134	104	133	130	141**	118	123	134
500 ppm	92	101	138	104	128	130	136**	128	134*	143
800 ppm	103	86**	146	95*	140**	120*	146**	121	133*	126

*: P<0.05 **: P<0.01

Table 5.5-04: Mean total protein (Tprot) and albumin (Alb) concentrations (g/dl)

Groups	W13		W26		W52		W78		W104	
	Tprot	Alb	Tprot	Alb	Tprot	Alb	Tprot	Alb	Tprot	Alb
Males										
control	7.2	2.9	7.0	2.9	7.2	2.9	7.1	2.7	7.1	2.6
25 ppm	7.1	3.0	6.9	2.9	7.2	3.0	7.1	2.8	7.3	2.5
125 ppm	7.1	2.9	6.9	2.8	7.2	2.9	7.1	2.7	7.1	2.4
500 ppm	6.9	3.0	6.7	2.9	6.9	2.9	6.9	2.7	7.1	2.6
800 ppm	7.1	3.0	7.2	2.9	7.0	2.9	6.8	2.6	6.8	2.5
Females										
control	7.5	3.4	8.0	3.7	8.0	3.9	7.6	3.3	8.0	3.3
25 ppm	7.4	3.5	8.0	3.7	7.8	3.7	7.3	3.2	8.1	3.5
125 ppm	7.3	3.4	7.6	3.5	7.6*	3.7	7.5	3.3	7.4*	3.1
500 ppm	7.3	3.3	7.5	3.3*	7.7*	3.6	7.3	3.2	7.5*	3.1
800 ppm	7.1*	3.3	7.6	3.4*	7.6*	3.6	7.1**	3.2	7.6*	3.2

*: P<0.05 **: P<0.01

There were no treatment-related changes in organ weights. No treatment-related macroscopic changes were noted.

At termination there was a dosage-related statistically significant increase in the incidence and degree of eosinophilic hepatocytes in male rats at 500 and 800 ppm. There was an increased incidence of ballooned cells in the liver of males at 125, 500 and 800 ppm. Comment: *This could represent an exacerbation of an age-related finding according to the author.*

Table 5.5-05: Incidence of eosinophilic hepatocytes and ballooned cells in decedent and terminal sacrificed males

	Dose levels in ppm				
	0	25	125	500	800
Total males examined	60	60	60	60	60
Eosinophilic hepatocytes: Total	21	28	21	**35**	**32**
Minimal	20	18	13	17	15
Moderate	1	6	4	**11**	7
Marked	0	4	4	**7**	**10**
Ballooned cells	12	12	**24**	**20**	**25**

There was no evidence of any treatment-related neurological lesions at the interim sacrifice (after 52 weeks of treatment) or at termination. There was no evidence of a tumorigenic or carcinogenic effect at any dosage level in this study.

Comments from the former RMS Sweden

There was no sign of oncogenic potential of deltamethrin in this study. No NOEL for male and female rats was determined in this study due to minor changes in biochemical parameters (changes in plasma electrolytes and reduced plasma cholesterol) noted in males and females receiving deltamethrin at 25 ppm and above. The NOAEL for male rats was 25 ppm (1 mg/kg bw/day) based on minor hepatotoxicity (increased incidence of ballooned cells in the liver) noted in males receiving deltamethrin at 125, 500 and 800 ppm. Additionally, clinical signs (uncoordinated movements of limbs and unsteady gait) (500, 800 ppm), increased incidence and degree of eosinophilic hepatocytes (500, 800 ppm), decreased body weight gain (500, 800 ppm) and reduced food consumption (500, 800 ppm) and minor changes in haematological parameters (reduced lymphocyte counts (125, 500, 800 ppm), reduced white cell counts (800 ppm), reduced haemoglobin concentration (800 ppm)) were noted for males. The NOAEL for female rats was 500 ppm (30 mg/kg bw/day) based on clinical signs (uncoordinated movements of limbs and unsteady gait noted in females receiving deltamethrin at 800 ppm). Reduced body weight gain during the first week of treatment (800 ppm), reduced food consumption during the first week of treatment (500, 800 ppm), minor changes in haematological parameters (reduced lymphocyte counts and white cell counts (125, 500, 800 ppm) were also noted for females. The study follows OECD guideline no 453. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Report:	KCA 5.5/02; [redacted] 1980; M-093417-01-1
Title:	RU 22974: Two year oral toxicity and carcinogenicity study in rats.
Report No.:	A20243
Document No.:	M-093417-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

Experimental design

Deltamethrin (purity 97-98%) was suspended in corn oil and administered in the diet to groups (90 male and 90 female rats/group) of [redacted] rats at respective concentrations of 2, 20 and50 ppm for 2 years (the concentrations corresponded to a dose rate of 0.1, 0.8 and 2.1 mg/kg bw/day for males and 0.1, 1.1 and 2.8 mg/kg bw/day for females). Control group 1 consisted of 90 animals/sex which received corn oil only. Additional, sixty male and sixty female rats were used in a second com oil control group (control group 2). Interim sacrifices of 10 rats/sex/group were conducted for all groups except for the second control group at 6, 12 and 18 months on study. The remaining rats from each group were sacrificed after 24 months.

The study complies with most requirements from the OECD guideline 453 revised in 2009, except that a reduced list of organs were examined histopathologically (epididymides and seminal vesicles not mentioned).

Results

Survivals were similar for control and treated rats. Survival at termination was between 50% and 63% for male rats and between 50% and 67% for female rats. No changes in general behaviour and appearance considered to be related to compound were observed.

Statistically significant lower mean body weights were noted for males of the 50-ppm dosage level as compared to the mean values for the control groups. Statistically significant lower body weight was noted for females of the 50-ppm dosage level only at 26, 65 and 78 weeks of study. Food consumption was similar for treated animals and controls.

Ophthalmoscopic, haematologic and urinalysis findings were similar for control and treated rats.

At 6 months a decrease in mean serum glutamic-pyruvic transaminase activity (ALT) values was noted for males and females of the 20 and 50 ppm dosage levels ($p<0.01$). Inorganic phosphorus mean values for these animals were also slightly lower than control values at 6 months (statistically significant only for females).

Statistically significant increased mean uterus weight (absolute and relative values), adrenals (absolute and relative values), thyroid (absolute value) and pituitary (absolute value) were noted for females fed deltamethrin at 50 ppm at 6 months. Statistically significant increased mean testis weight (relative value) was noted for males fed deltamethrin at 50 ppm at 6 months. Statistically significant decreased mean thyroid weight (absolute value) was noted for males fed deltamethrin at 50 ppm at 12 months. Comment: *At terminal sacrifice statistically significant increased mean testes weight (relative value) in males at 50 ppm was the only noted effect on organ weights.*

No compound-related gross necropsy observations were seen in any of the experimental groups.

The incidence and/or relative severity of axonal degenerations in sciatic, tibial and/or plantar nerves among male and female rats at 20 and 50 ppm dietary levels sacrificed after 18 months were increased. At termination, the incidence and/or relative severity of such observations, were similar for both controls and experimental groups.

There was no evidence for a carcinogenic effect of deltamethrin in rats. The incidence of testicular interstitial cell adenoma was increased in males receiving 50 ppm deltamethrin in the diet (7.8%) compared to control group 1 (0%) (see table 5.5-06). However, interstitial cell tumour (adenoma) occurred with almost equal frequency in the second control group (8.3%) as in the high dosage group. Furthermore, historical control incidence of interstitial cell tumours in chronic studies performed on CD-I mice for animals delivery dates between 1975 and 1979, ranged between 0 and

22.2% (mean value: 8.8%). Therefore, the increased incidence of testicular interstitial cell adenoma in males at the 50-ppm dietary level was considered spontaneous and unrelated to the administration of the test substance.

Table 5.5-06: Incidence of testicular lesions

Dose levels (ppm)	0 (control 1)	2	20	50	0 (control 2)
No examined	90	90	90	90	60
Testicular degeneration	12	13	13	20	10
Mineralization	2	3	6	2	-
Necrosis, seminiferous tubule	1	-	-	-	-
Arteritis, chronic	10	9	12	18	14
Interstitial cell hyperplasia	2	2	-	4	2
Interstitial cell adenoma	—	1	1	7	6
Malignant lymphoma	—	—	-	-	1

Table 5.5-07: Incidence of axonal degeneration at 18-month interim sacrifice of a small no of animals (7-10 animals/sex/group)

Dose level (ppm)	Sciatic	Tibial nerve	Plantar nerve
0	10% (m) non (F)	40% (m) and 14% (F)	none
2	10% (m) non (F)	50% (m) and 20%	none
20	50% (m) 10 (F)	89% (m) and 40% (F)	none
50	80% (m) 10 (F)	100% (m) and 25% (F)	33% (m) and 13% (f)

Comments from the former RMS Sweden

There was no sign of oncogenic potential in deltamethrin in this study. Minor changes in biochemical parameters were noted in rats of the 20- and 50 ppm dosage levels at 6 months on study. The NOAEL for male and female rats was 20 ppm (0.8 and 1.1 mg/kg bw/day for males and females, respectively) based on decreased body weight and variations in mean weight in various organs noted in rats of the 50-ppm dosage level. The increased incidence and/or relative severity of axonal degenerations in nerves seen at 18 months in this study was only noted at interim sacrifice of a small number of animals (7-10 rats/sex/group). At termination of the study the incidence and/or relative severity of such observations were generally similar for both controls and experimental groups. Furthermore, it was impossible to repeat the findings in a later performed 2-year feeding study on rats where the dose levels were much higher compared to this study (see [redacted], 1995). This indicates that the lesions were not compound related. The study follows OECD guideline no 453. There is a statement of compliance with GLP but no Quality Assurance inspections were made. The study seems to be of acceptable quality.

Report:	KCA 5.5/03; [redacted]; 1980; M-093412-01-1
Title:	RU 22974. Two Year Oral Toxicity and Carcinogenicity Study in Mice
Report No.:	A20242
Document No.:	M-093412-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 96-100%) was suspended in corn oil and administered in the diet to groups (80 male and 80 female mice/group) of [redacted] mice at respective concentrations of 1, 5, 25 and 100 ppm for 2 years. The concentrations corresponded to a dose rate of 0.1, 0.6, 3.1 and 12 mg/kg bw/day for male mice and 0.1, 0.8, 3.8 and 15 mg/kg bw/day for female mice. The controls (80 animals/sex) received the vehicle only. Additionally, sixty male and sixty female mice were used in a second corn oil control group. Interim sacrifices of 10 mice/sex/group were conducted for all groups except for the second control group at 12 and 18 months of study. The remaining mice were sacrificed after 24 months.

The study complies with most requirements from the OECD guideline 453 revised in 2009, except that a reduced list of organs were examined histopathologically (epididymides and seminal vesicles not mentioned), prothrombin time, activated partial thromboplastin time, creatinine, albumin and total cholesterol were not determined. Although the study was performed prior the GLP regulations were in place, a statement from the study director is given in the report confirming that GLP rules were applied.

Results

Survival rates were similar for control and treated mice. Survival at termination was between 47% and 53% for male mice and between 40% and 58% for female mice. No signs of overt toxicity were observed among the treated mice.

A slight decrease in mean body weights were noted for the 100 ppm male and female dosage levels. The depression of body weight was 8% for males and 6% for the females. No compound-related effects were observed with respect to food consumption.

There were no treatment-related effects on any haematology, clinical chemistry or urinalysis parameter.

Statistically significant decreased mean kidney weight (absolute value) and increased mean adrenal weight (absolute and relative) were noted for males fed deltamethrin at 100 ppm at 12 months. Statistically significant decreased mean ovary weight (absolute and relative values) was noted for females fed deltamethrin at 100 ppm at 12 months. Statistically significant increased mean thyroid weight (absolute and relative values) was noted for females fed deltamethrin at 100 ppm at 18 months. Comment: *At terminal sacrifice statistically significant increased mean heart weight (relative value) in males at 100 ppm was the only effect noted on organ weights.*

No treatment-related gross- or microscopic changes were observed.

There was no evidence for a carcinogenic effect of deltamethrin. The incidence and type of tumours observed was consistent with those normally expected in this strain of mouse.

Comments from the former RMS Sweden

There was no sign of oncogenic potential of deltamethrin in this study. A slight decrease in mean body weight (6-4% depression only in males and females,respectively) and variations in mean organ weights

were noted at the highest dose level (100 ppm). Due to that, the NOAEL for male and female mice was >100 ppm (12 mg/kg bw/day for males and 15 mg/kg bw/day for females). The study follows OECD guideline no 453. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections.

Report:	KCA 5.4.3/02; [redacted]; 1995; M-149308-01-1
Title:	Deltamethrin technical: 97-week carcinogenicity study by oral route (dietary admixture) in mice.
Report No.:	A70820
Document No.:	M-149308-01-1
Guideline(s):	JMAF: 59 Nohsan No.4200, (Jan.1985); OECD: 451, (May 1981); USEPA (=EPA): Subdivision F, 83-2, (Nov.1984)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Swiss mice (50 male and 50 female mice/group) of the [redacted] BR strain were administered deltamethrin (purity 98-100 %) in dietary mixtures at concentrations of 10, 100, 1000 or 2000 ppm for at least 97 weeks. The concentrations corresponded to a dose rate of 2, 16, 155 and 315 mg/kg bw/day for males and 2, 20, 189 and 395 mg/kg bw/day for females. The controls (50 animals/sex) received the diet only.

The study follows the OECD guideline 451, revised in September 2009.

Results

Mortality at termination (week 97 inclusive) was between 36% and 40% for male mice and between 44% and 56% for female mice. In the 2000 ppm group emaciation and dyspnea were noted in males and females at an incidence which was slightly higher compared to the control group. Comment: *In most cases, these signs were noted a few weeks before death, in animals killed prematurely or found dead.*

Statistically significant lower body weight gain was noted for males of the 2000 ppm group compared to the controls, mainly during the first year of the treatment period. The food consumption of the treated animals of all groups was similar to that of controls.

There were no treatment-related effects on haematology.

There were no statistically significant differences in organ weights.

Ulceration together with cellulitis in the skin of different part of the body, including the ears was found with higher frequency in the males given 1000 ppm and in the animals of both sexes given 2000 ppm. Comment: *These findings were considered to be an indirect consequence of the test substance on pain sensors.* Otherwise, no treatment-related gross- or microscopic changes were observed. There was no evidence for a carcinogenic effect of deltamethrin. The incidence and type of tumours observed was consistent with those normally expected in this strain of mouse.

Comments from the former RMS Sweden

There was no sign of oncogenic potential of deltamethrin in this study. The NOEL for male mice was 100 ppm (16 mg/kg bw/day) based on skin lesions noted in males receiving deltamethrin at 1000

ppm and above. Clinical signs (dyspnea and emaciation) and reduced body weight gain were noted in males fed deltamethrin at 2000 ppm. The NOEL for female mice was 1000 ppm (189 mg/kg bw/day) based on clinical signs (dyspnea and emaciation) and skin lesions noted in females fed deltamethrin at 2000 ppm. The study follows OECD guideline no 451. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

CA 5.6 Reproductive toxicity

No new studies have been performed since the last EU review.

A justification for not repeating the conventional vertebrate studies conducted according to the OECD guidelines in place at the time of first submission is provided below.

BCS conducted a two-generation reproductive toxicity study following OECD test guideline 416 (1983). In addition, two developmental toxicity studies in the rat followed OECD test guidelines 414 (1981). Although both guidelines have been revised since then to incorporate additional endpoints, respectively in the reproduction toxicity (estrous cycle, sperm enumeration, motility and morphology, and sexual maturation evaluations) and developmental toxicity (longer period of administration), BCS did not repeat these studies because no hint of reprotoxicity has never been seen in any available study. In addition, recommendations laid down in the Animal Testing Directive (2013) and Recital 40 of the PPP Regulation stating that "tests on vertebrates should be undertaken as a last resort" (and may under no circumstances be duplicated) were followed. Furthermore, the sexual maturation has been evaluated in the developmental neurotoxicity study.
With regard to the developmental toxicity studies, the test guideline OECD 414 “includes assessment of maternal effects as well as death, structural abnormalities, or altered growth in the foetus”. Several authors have reported that most developmentally susceptibilities occur from implantation to the end of organogenesis period. This explains why, according to the OECD 414 (1981), the test item is administered to the pregnant animal during the period of major organogenesis. This period starts after implantation through the closure of the hard palate, i.e. approximately days 6/7 to 15/17 in the rodent, and days 6 to18 in the rabbit. Any effect, death, structural abnormalities, or altered growth occurring during this period is easily detectable at cesarean section or during fetal examination. In addition, such design offers the use of higher dosages that are better tolerated by animals thereby maximizing the chance to pick up an effect.
After organogenesis, during the late gestational period, the foetus is less sensitive to structural alterations but functional maturation can be affected by treatment. However, as specified in the introduction section of the revision of the TG (2001), “functional deficits are not a part of this Guideline”. They may be tested for in a separate study like the rat two-generation reproductive toxicity study and the developmental neurotoxicity study. Both studies are available for deltamethrin. Such studies can also identify late effects on the pregnant test animal and the growth of the developing animal as measured by body weight determination during gestation, at birth and during lactation and viability index.
Therefore the sequence of treatment periods in the developmental toxicity and two generation toxicity studies do not leave any phase of the reproductive process uncovered. A new rabbit developmental toxicity study was performed in 2001 following the current guideline.

In conclusion, it is considered that the regulatory requirement to provide information concerning the effects of prenatal exposure on the pregnant test animal and on the developing organism, including assessment of maternal effects as well as death, structural abnormalities, or altered growth in the foetus is fulfilled with the results of studies M-149348-01-1, M-149353-01-1 and M-204103-01-1 and that additional animal testing is not required. (D. Beltrame and G. Mazue. Reproductive toxicology guidelines: comparison and application. Ann. Ist. Super. Sanita, 29, 3-14, 1993 - De Sesso, J.M., 1997. Comparative Embryology. In: Handbook of Developmental Toxicology, Hood, R.D. (Ed.). CRC Press, Boca Raton, USA., pp: 111-174. - Gilbert SF. Developmental Biology (9th Edition). - Hood RD - Developmental and Reproductive Toxicology: A Practical Approach, Second Edition, 2005.)

Deltamethrin did not affect reproduction in a two generation study ([redacted]; 1992; M-149348-01-1) in rats fed deltamethrin at concentrations from 5 to 320 ppm. The parental NOEL was 80 ppm (4.2 mg/kg/day) based on mortalities, clinical signs (ataxia, hyperactivity, alopecia, splayed limbs, vocalization, salivation, impaired righting reflex, urine-stained abdominal fur, body surface staining), reduced body weight and histopathological changes (gastric erosion) noted in adult rats at 320 ppm. The NOEL for reproduction was 320 ppm (18.3 to 43.8 mg/kg/day). The offspring NOEL was 80 ppm based on reduced pup weights, increased pup deaths (F1 generation) and reduced lactation index (F1 generation) noted at 320 ppm. Sperm analysis, oestrous cycle and sexual maturation were not assessed in this study. Sexual maturation has been evaluated in the Developmental Neurotoxicity Study. However, for sperm analysis and oestrous cycle parameters, no additional study was performed because of animal welfare consideration and because there is no hint of impaired reproduction in any of the toxicity studies performed with deltamethrin. It is considered that a new study wouldn't bring any significant information.

Deltamethrin did not induce any developmental toxicity in rats, mice or rabbits.

In a first study performed by [redacted] in 1977 (M-093444-01-1) in rats, mice and rabbits, the dose levels were not high enough to induce significant effects.

In the rabbit development study ([redacted]; 2001; M-204103-01-1), deltamethrin did not induce any embryotoxicity, foetoxicity and teratogenicity. The maternal NOEL was 10 mg/kg bw/day based on slight reduced body weight and food intake at 32 mg/kg/day. There were no treatment-related effects in foetuses at any dose level. The foetal NOEL was 32 mg/kg/day. In a previous rabbit developmental study ([redacted]; 1990; M-149350-01-1), mortality in the dams and retarded ossification in the pups were observed at 100 mg/kg/day.

In rat development studies ([redacted] 1979; M-094154-01-1 and [redacted]; 1990; M-149353-01-1), deltamethrin did not induce any embryotoxicity, foetal toxicity and teratogenicity. Treatment-related effects in dams consisted of mortality ([redacted]; 1990; M-149353-01-1), clinical signs and reduced body weight. On that basis the dose levels of 2.5 and 3.3 mg/kg bw/d were considered to be the maternal NOAELs of the respective studies ([redacted] 1979; M-094154-01-1 and [redacted]; 1990; M-149353-01-1). Treatment-related effects in foetuses were limited to the high dose treated group of Kavlock's study. In this study, some of the dams were allowed to give birth and – those remaining on dose - to raise pups during lactation until day 15 post-partum. Pups were afterwards reared on untreated diet until day 42 post-partum. Effects on pup body weight during pre-weaning, disappeared upon the cessation of dosing. Additional neurological investigations of locomotor activity, open field observation and righting and auditory startle reflexes showed that deltamethrin did not affect the normal development of these foetuses at any dose level. On that basis, the slight and transient early changes in body weight

reported in foetuses of the high treated group were considered to be of no toxicological relevance. On that basis, the dose level of 5 mg/kg bw/d was considered to be the foetal NOEL.

Although the most recent rat developmental toxicity study ([redacted] 1990; M-149353-01-1) does not strictly follow the current guideline (administration from gestation day 6 to gestation day 15 versus 20), it is not considered necessary to repeat this study. No hint of developmental toxicity was observed in any toxicity study performed with deltamethrin. A longer period of administration would have not provided any additional valuable information. This is confirmed by the results of the developmental toxicity study ([redacted] 2013; M-270180-03-1, see also CA 5.7) where the animals were administered during the gestation period.

In a mouse developmental toxicity study ([redacted] 1979; M-094154-01-1) where deltamethrin was administered in corn oil by gavage at doses from 3 to 12 mg/kg/day, maternal reduced body weight gain was noted at 3 mg/kg/day and all subsequent dose levels and maternal clinical signs (convulsions) were observed at 6 mg/kg/day and all subsequent dose levels. In offsprings, an increased incidence in supernumerary ribs was observed in all treated groups but with no dose-relationship.

Table 5.6-01: Summary of reproductive and developmental toxicity studies of deltamethrin (studies in the baseline dossier in gray)

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
Rat 2 generation [redacted], 1992 M-149348-01-1 0, 5, 20, 80, 320 ppm	Parental: 80	4.2	320	18.3	Parents: mortalities in F P1 generation and both M & F F1 generation, neurological signs, ↓BW & FC
	Reprod: 320	18.3	> 320	>18.3	Reproduction: no treatment-related effects
	Offspring: 80	4.2	320	18.3	Offspring ↑mortalities, ↓ lactation index in P1, ↓ pup weights
Rat developmental toxicity [redacted], 1977 M-093444-01-1 0, 0.1, 1, 10 mg/kg/day	Dams: 10		>10		Slight retarded BWG in dams
	Pups: 10		>10		No treatment-related effects
Rat developmental toxicity [redacted] 1978 M-094154-01-1 0, 1.25, 2.5, 5 mg/kg/day	Dams: 2.5		-	5	Clinical signs (mild salivation), ↓BWG (20%)
	Pups:5			>5	No treatment-related effects

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
Rat developmental toxicity [redacted], 1990 M-149353-01-1 0, 1, 3.3, 7, 11 mg:kg/day		Dams: 3.3 Pups: 11		7 >11	Dams: mortalities, ↓BWG, neurological signs (convulsions, salivation, ↑sensitivity to external stimuli) Pups: No treatment-related effects
Rabbit developmental toxicity [redacted], 1977 M-093444-01-1 0, 1, 4, 16 mg/kg/day		Dams & pups: 16		>16	No treatment-related effects
Rabbit developmental toxicity [redacted], 1990 M-149350-01-1 0, 10, 25, 100 mg/kg/day		Dams & pups: 25		100	Mortality in dams and retarded ossification in pups
Rabbit developmental toxicity [redacted], 2008 M-204103-01-1 0, 3, 10, 32 mg/kg/day		Dams: 10 Pups: 32		32 >32	↓BW & FC No treatment-related effects
Mouse developmental toxicity [redacted], 1977 M-093444-01-1 0, 0.1, 1, 10 mg/kg/day		Dams & pups: 10		>10	No treatment-related effects
Mouse developmental toxicity [redacted] 1978 M-094154-01-1 0, 3, 12 mg/kg/day		Dams: 3 Pups: 12		3 >12	↓BWG (18%) ↑ Supernumerary ribs in offspring but not dose-related

Comparison with classification and labelling criteria:

The reproduction potential of deltamethrin was evaluated in rats, mice and rabbits.

In the rat two generation reproduction study, dietary exposure to deltamethrin at concentration as high as 320 ppm did not affect mating performance or fertility of male or female rats from both generations. Mortality was observed at 320 ppm in one female from the P1 generation and 17 males and 19 females from the F1 generation. The males and females from the F1 generation died within 8 days after weaning. Marked neurological signs were also observed at this dose level. Increased pup deaths was observed in the P1 generation on days 8 to 14 post-partum with a significantly reduced lactation index, but not observed in the F1 generation.

In the developmental toxicity studies, deltamethrin did not affect the number of corpora lutea, implantation sites or litter size, or the development of the foetuses in neither the rat, the rabbit or the mouse. In the mouse, the occurrence of supernumerary ribs was not dose-related; the delayed ossification of the sternebrae noted in foetuses of dams receiving DLT at 1 and 10 mg/kg/day was within normal limits of historical control animals and not seen in the study of Kavlock where the animals were exposed up to 12 mg/kg.

In the rabbit, retarded ossification and an increased incidence of foetuses with 27 pre-sacral vertebrae were observed at 100 mg/kg/day in the [redacted] study M-149350-01-1, where the rabbits were administered deltamethrin from GD7 to 19. Very little toxicity was observed in the does except one mortality at GD27 and no effect on viability of the pups or structure (no malformations). However, only a reduced number of litters or foetuses were observed in each group, in this study and decreased mean foetal weight was due to slight increase litter size in the treated groups. Therefore the slight effect of foetal weight could explain the retarded ossification. A more recent study (M-204103-01-1), where 24 does per groups were exposed to deltamethrin from GD6 to 28, confirmed there were no effects of treatment on viability or structure but did not show any effect on the incidence of skeletal variations (double staining in this new study), even though the exposure period was longer and therefore more likely to induce foetal toxicity. Therefore, it is considered that as the second study, with the more precautionary design, did NOT confirm the skeletal variations, that these original un-repeatable findings are not considered toxicologically relevant.

It can be concluded that deltamethrin is not reprotoxic as it has no potential to affect mating performance or fertility in the rat, the number of corpora lutea, implantation sites or litter size in the rat, the rabbit and the mouse. Deltamethrin has no embryotoxic or teratogenic potential. In conclusion deltamethrin does not warrant classification for any reproductive toxic effects.

Conclusion on classification:

CLP Regulation: **No classification**

CA 5.6.1 Generational studies

The rat 2 generation reproduction study was presented and evaluated during the EU process for Annex I listing. A copy of the summary performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Report:	KCA 5.6.1/01; [redacted]; 1992; M-149348-01-1
Title:	Reproductive effects of deltamethrin administered orally in diet to Crl: CD BR VAF/Plus rats for two generations. (Vol.1/4).
Report No.:	A70863
Document No.:	M-149348-01-1
Guideline(s):	USEPA (=EPA): 83-4
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Rats ([redacted]) were administered deltamethrin (purity 99.7%) in the diet at concentrations of 5, 20, 80 and 320 ppm. Each group consisted of 30 rats per sex. The controls (30 animals/sex) received the diet only. The first generation (P1) rats and the second generation (Fl) rats were given dietary levels of the test substance for approximately 12 weeks before the three-week cohabitation period. Mating was verified by detecting a vaginal sperm plug (day 0 of gestation). P1 generation male rats were sacrificed approximately three weeks after completion of the cohabitation period. P1 generation female rats had continual access to the test diets through the cohabitation and gestation until scheduled sacrifice occurred on day 21 of lactation. The Fl generation male and female rats had continual access to the test diets through the cohabitation and gestation until scheduled sacrifice after production of the F2 generation litters. Complete gross necropsies were performed on all P1 and Fl generation rats following sacrifice. The female rats were also examined for the presence of implantation sites. The Fl generation pups not selected for continual observation and all F2 generation pups were killed and examined for gross lesions on day 21 postpartum. Necropsy of all pups included an examination of the brain for hydrocephaly. Histopathological examination of all reproductive and target organs was performed for all control and high dosage groups. All gross lesions were also examined for histopathology.

Protocol following the current OECD guideline N°416 on two generation reproduction study except that there were no evaluation on the estrous cycles, sperm enumeration, motility and morphology or sexual maturation. However, sexual maturation was assessed during the rat developmental neurotoxicity study. No additional study was performed, as there was no hint of reproductive toxicity potential in any available regulatory study.

Results

One P1 generation female rat, 17 Fl generation male rats and 19 Fl generation female rats receiving 320 ppm deltamethrin in the diet died. Most of the Fl generation rats were found death within eight days after weaning. Ataxia, hyperactivity, alopecia, splayed limbs, vocalization, excess salivation, impaired righting reflex, urine-stained abdominal fur and a red brown and/or yellow substance present at various locations on the body were noted in many 320 ppm group F1 rats. **Ataxia and hypersensitivity were observed in P1 females during lactation.**

Significantly reduced body weights, body weight gain and food consumption were noted for P1- and Fl generation male and female rats of the 320 ppm group.

The absolute weights of ovary (the right), nongravid uterus and pituitary in the 320 ppm group P1 generation female rats, and ovary (the right) and nongravid uterus in the 320 ppm group Fl generation female rats, and the epididymides and testes in the 320 ppm group Fl generation male rats were significantly reduced as compared to the control values. Comment: *These effects were considered to*

reflect the significantly reduced terminal body weights because the organ weight to body weight ratios were not significantly reduced in the 320 ppm group whereas the organ weights to brain weight ratios were significantly reduced.

Necropsy observations which were attributable to the test substance were noted in rats that died only. One of the Fl generation male rats in the 320 ppm group which died had gastric erosions and another had large adrenals. Gastric erosions were also noted for the P1 generation female rats which died in the 320 ppm group. Comment: *Erosions in the stomach were also observed at the 1000 ppm concentration in the dosage-range evaluation for deltamethrin.*

The reproductive organs of the Fl generation male and female rats in the 320 ppm dosage group were small. Comment: *This effect was considered to be due to immaturity as most of these rats died very early in the study.*

Exposure to deltamethrin at concentrations as high as 320 ppm in the diet did not affect mating performance or fertility of the P1-or Fl generation male and female rats.

Pup deaths were significantly increased for the P1 generation pups in the 320 ppm group on days 8 to 14 postpartum, and the lactation index was significantly reduced.

Pup weights per litter of the Pl-and Fl generation in the 320 ppm group were less at birth and the pups had significantly reduced body weights.

There were no other differences of biological significance among the groups in pup viability, sex ratios or clinical or necropsy observations.

Comments from the former RMS Sweden

Deltamethrin did not affect reproduction in rats in this study. NOEL for deltamethrin in adult male and female rats was 80 ppm (the average consumed dosages ranged from 4.2 to 12.4 mg/kg bw/day in the periods evaluated in this study) based on death, clinical observations, reduced body weight, reduced food consumption and gastric erosion noted in animals of the 320 ppm level. NOEL for deltamethrin in the offspring was 80 ppm based on increased pup deaths, a reduced lactation index and reduced body weight noted in animals of the 320 ppm dosage level. The study follows OECD guideline no 416. It was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

CA 5.6.2 Developmental toxicity studies

These studies were presented and evaluated during the EU process for Annex I listing. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

Results obtained in the rat

Report:	KCA 5.6/02; [redacted]; 1977; M-093444-01-1
Title:	RU 22974. Teratological Study in Mouse - Rat - Rabbit
Report No.:	A20256
Document No.:	M-093444-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**
***Annex data point due to first Annex I listing**	

Experimental design

Deltamethrin (purity not specified) dissolved in sesame oil was administered by gavage to mated female Sprague-Dawley rats (24 animals/group) at dose levels of 0 (sesame oil control), 0.1, 1 and 10 mg/kg bw/day during days 6-18 of gestation. Mating (accomplished naturally) was verified by detecting spermatozoa in the vaginal smears (rat). In all cases the day of observation was considered as day 0 of gestation. Rats were sacrificed on day 21 of gestation. Dams and foetuses were then examined. In the rat, 12 dams receiving deltamethrin at 10 mg/kg bw/day and 12 dams receiving the vehicle alone were allowed to deliver normally and raise their litters for weaning. At 28 days dams and pups were sacrificed and examined for gross pathological changes.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses, running a concurrent positive control group and required observations. However few deviations are observed such as the period of administration which was from GD6 to 18, the purity of deltamethrin which is not specified, the housing conditions in the experimental room which are not mentioned. The study was not conducted under GLP, but the data are robust enough.

Results

Rat

No maternal mortality or clinical signs of toxicity were observed during the study.
Reduced maternal bodyweight gain (13%) was noted in animals receiving deltamethrin at the dose level of 10 mg/kg bw/day when compared to the animals in the control group.
No effects were observed on the number of implantation sites or fetal mortality. Statistically significant delayed ossification of the sternebrae was noted in foetus of dams receiving deltamethrin at 10 mg/kg bw/day (30%) when compared to the control group animals (17%) (the value was within normal limits for historical control animals). Survival, bodyweight gain and behaviour of the newborn was unaffected by treatment. Internal examination of weaning rats revealed normal morphology.

Comments from the former RMS Sweden

NOEL for maternal toxicity in rats was 1 mg/kg bw/day based on reduced maternal bodyweight gain (13%) in animals receiving deltamethrin at the dose level of 10 mg/kg bw/day. NOAEL for developmental toxicity in rats was 10 mg/kg bw/day. Delayed ossification of the sternebrae was noted in foetuses of dams receiving deltamethrin at the dose level of 10 mg/kg bw/day. NOEL for neonatal toxicity in rats was ≥10 mg/kg bw/day.

Report:	KCA 5.2/01; [redacted] 1979; M-094154-01-1
Title:	Toxicity studies with decamethrin, a synthetic pyrethroid insecticide.
Report No.:	A20968
Document No.:	M-094154-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity not specified) dissolved in corn oil was administered by gavage to Sprague-Dawley rats (29-37 animals/group) at doses of 0 (corn oil control), 1.25, 2.5 and 5 mg/kg bw/day during days 7-20 of gestation. Day 1 of pregnancy was recorded upon demonstration of a copulatory plug. Rats were sacrificed on day 21 of gestation. Dams and foetuses were then examined. An addition group of pregnant rats (12-14 animals/group) was housed individually and gavaged with dosages of either 0, 2.5 and 5 mg/kg bw/day from day 7 of gestation to day 15 of lactation. Litters from these dams were reduced at birth to four individuals of each sex. Besides growth and survival, the following parameters were evaluated in pups: eye opening, startle and air righting reflexes, circular open field test in females at 6 weeks of age.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses, running a concurrent positive control group and required observations. However few deviations are observed such as the purity of the test substance is not specified and the housing and feeding conditions which are not mentioned. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed). The study seems to be of acceptable quality.

Results

Rat

No dose-related occurrences of maternal mortality were observed. Clinical sign (salivation) was noted for dams in the high dosage group (5 mg/kg bw/day). Dose-related statistically significant reduced maternal body weight gain were noted.

No effects were observed on the number of implantation sites, fetal mortality, fetal weight or number of sternal and caudal ossification centers. No effect on parturition, litter size or pup viability were noted. Neonatal weights at birth were similar for all groups, but a dose-related depression in growth was observed during the pre-weaning period.

Comments from the former RMS Sweden

No NOEL for maternal toxicity was determined for rats due to reduced maternal body weight gain. NOEL for developmental toxicity in rats was >5 mg/kg bw/day. The NOEL for neonatal toxicity in rats could not be determined due to reduced neonatal bw gain at 1.25 mg/kg bw/day. The reference is a published article (J. Env. Path. and Tox. 2:751-765, 1979) which consists of a summary of toxicity studies on deltamethrin conducted by the E.P.A, United States. No raw data were available. The study seems to follow OECD guideline no 414 except for some minor deviations. The purity of the test substance was not specified and there were lack of data concerning housing or feeding conditions. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed). The study seems to be of acceptable quality.

Report:	KCA 5.6.2/01; [redacted]; 1990; M-149353-01-1
Title:	Developmental toxicity study of deltamethrin in rats.
Report No.:	A70869
Document No.:	M-149353-01-1
Guideline(s):	OECD: 414; USEPA (=EPA): 83-3
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 99.4%) suspended in corn oil was administered orally by gavage to mated female Sprague-Dawley derived [redacted] rats (25 animals/group). Dosing was performed from day 6 to 15 of gestation. Mating (accomplished naturally) was verified by detecting a copulatory plug (day 0 of gestation). Initially, dosage levels of 0 (corn oil control), 1, 3.3 and 11 mg/kg bw/day were used. Due to excessive toxicity at the high-dose level, a fourth treatment level at 7 mg/kg bw/day was added, and due to unacceptable concentration analyses one additional control (corn oil) and two additional treatment groups at dosage levels of 1 and 3.3 mg/kg bw/day were added (25 animals/group). The rats were killed on day 20 of gestation and dams and foetuses were then examined.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design and required observations. However few deviations are observed such as the initial dose selection which caused 56% mortality at the highest dose (however another group treated at a lower dose level of 7 mg/kg/day was added into the study), the period of administration which is between GD6 to 15 with a final sacrifice on GD20, the volume of administration which is not mentioned, the lack of food consumption data and the lack of another control group than the vehicle control group. However the data obtained in this study seem reliable enough.

Results

Maternal toxicity was evidenced by treatment-related deaths and moribund conditions at 7 and 11 mg/kg bw/day dosage levels (1/25 and 14/25 for the groups, respectively). Clinical findings observed among these and animals which survived to scheduled sacrifice included moribundity, convulsions, anogenital staining, increased salivation, sensitivity-to-external stimuli, and body surface staining. Increased incidence of piloerection was the only effect noted for the 3.3 mg/kg bw/day level animals.

Reduced body weight gains were noted in dams receiving 7 and 11 mg/kg bw/day compared to the control animals. **From treatment initiation (gestation day 6) to gestation day 9, animals in the 7 mg/kg/day dosage group gained only half as much weight as the control group, and animals in the 11 mg/kg/day dosage group lost weight.**

No treatment-related embryotoxic or teratogenic effects were observed at any dosage level tested.

Comments from the former RMS Sweden

Based on this study, deltamethrin was not teratogenic in rats. NOEL for maternal toxicity was 3.3 mg/kg bw/day based on maternal death, clinical signs (convulsions, increased salivation, sensitivity-to-external stimuli, anogenital staining, body surface staining) and reduced body weight gain noted in dams receiving deltamethrin at 7 and 11 mg/kg bw/day. NOEL for developmental toxicity was >11 mg/kg bw/day. The study follows OECD guideline no 414 except for the fact that the highest dose level (11 mg/kg bw/day) was too high (caused 56% maternal deaths). According to the guideline the highest dose level should not cause more than 10% maternal deaths. There are also lack of food consumption data, and no other control group

than the vehicle control group was used in the study. There are no statements concerning GLP but the study was subjected to Quality Assurance inspections and seems to be of acceptable quality.

Results obtained in the rabbit:

Report:	KCA 5.6/02; [redacted]; 1977; M-093444-01-1
Title:	RU 22974. Teratological Study in Mouse - Rat - Rabbit
Report No.:	A20256
Document No.:	M-093444-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity not specified) dissolved in sesame oil was administered by gavage to mated female New Zealand White SPF rabbits (15 animals/group) at dose levels of 0 (sesame oil control), 1, 4 and 16 mg/kg bw/day during days 6-19 of gestation. In a complementary study 15 mated female New Zealand White SPF rabbits received deltamethrin by gavage at the dose level of 16 mg/kg bw/day during days 6-19 of gestation. Mating (accomplished naturally) was verified visually (rabbit). The day of observation was considered as day 0 of gestation. Rabbits were sacrificed on day 28 of gestation. Dams and foetuses were then examined.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses, running a concurrent positive control group and required observations. However few deviations are observed such as the number of animals which is too low in each group, the period of administration which was from GD6 to 19, the purity of deltamethrin which is not specified, the housing conditions in the experimental room which are not mentioned. The study was not conducted under GLP.

Results

Rabbit

No treatment-related occurrences of maternal mortality was observed. Two females receiving deltamethrin at the dose level of 16 mg/kg bw/day died during the complementary study. The animals had obvious signs of pneumonia. No clinical sign of toxicity was observed in the surviving animals.

Slight reduced maternal bodyweight gain (3-4%) was noted in treated animals compared to the animals in the control group.

Statistically significant increased total foetal loss was noted in animals receiving deltamethrin at the dose levels of 1 mg/kg bw/day (18.5%) and 4 mg/kg bw/day (15.5%) when compared to the control group animals (6.6%). Increased total foetal loss (not statistically significant) was also noted in animals receiving deltamethrin at the dose level of 16 mg/kg bw/day (10.2%). In the complementary study statistically significant increased total foetal loss (27.3%) was noted in the animals receiving deltamethrin at the dose level of 16 mg/kg bw/day compared to the control group animals (6.6%).

Statistically significant decreased foetal weight was noted in foetus of dams receiving deltamethrin at the dose level of 16 mg/kg bw/day when compared to the control group animals. Decreased foetal weight (not statistically significant) was also noted in foetuses of dams receiving deltamethrin at the dose level of 16 mg/kg bw/day in the complementary study when compared to the control group animals. Some malformations were noted in two foetuses of dams receiving deltamethrin at the dose level of 16 mg/kg bw/day. The incidence of these malformations (1-2%) was out of normal limits for historical control animals for the laboratory in question (0-0.6%). Hydrocephaly associated with brachignathia was noted in one of the two foetuses. Exencephaly associated with thoracogastrochisis, bilateral amelia and twistling of the umbilical cord round the neck was noted in the other one. These abnormalities were not observed in foetuses of dams receiving deltamethrin at the dose level of 16 mg/kg bw/day in the complementary study. In the complementary study, spina-bifida and shortened tail was noted in one foetus of dam receiving deltamethrin at the dose level of 16 mg/kg bw/day.

Comments from the former RMS Sweden

NOEL for maternal toxicity in rabbits was ≥16 mg/kg bw/day. No NOEL for developmental toxicity in rabbits was determined due to increased total foetal loss noted in animals receiving deltamethrin at the dose levels of 1 mg/kg bw/day (statistically significant), 4 mg/kg bw/day (statistically significant) and 16 mg/kg bw/day (not statistically significant). Statistically significant increased total foetal loss were also noted in animals receiving deltamethrin at the dose level of 16 mg/kg bw/day in the complementary study. Decreased foetal weight and malformations (one case of hydrocephaly associated with brachignathia, one case of exencephaly with thoracogastrochisis, bilateral amelia and twistling of the umbilical cord round the neck and one case of spina bifida and shortened tail) were also noted in some foetuses of dams receiving deltamethrin at the dose level of 16 mg/kg bw/day. The study follows OECD guideline no 414 except for some deviations. The purity of the test substance was not specified and there were lack of data concerning housing conditions. No other control group than the vehicle control group was used in the study. The studies on deltamethrin in mice and rats seem to be of acceptable quality. The study on deltamethrin in rabbits is not of acceptable quality due to the fact that the health condition of the experimental animals in the complementary study was questionable (two animals died because of pneumonia). Further on, there were insufficient pups produced to permit an evaluation of the teratogenic potential of deltamethrin. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed).

Report:	KCA 5.6.2/02; [redacted]; 1990; M-149350-01-1
Title:	Developmental toxicity study of deltamethrin in New Zealand white rabbits.
Report No.:	A70865
Document No.:	M-149350-01-1
Guideline(s):	USEPA (=EPA): 83-3 (b)
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

Deltamethrin (purity 99.4%) was administered by gavage to 1 control and 3 treatment groups of artificial inseminated New Zealand White SPF female rabbits (16 animals/group). Dosing was performed from day 7 to 19 of gestation. Dose levels were 0,10,25 and 100 mg/kg bw/day.

Carboxymethylcellulose (0.5%) was used as the vehicle and control. The rabbits were killed on day 29 of gestation and dams and foetuses were then examined.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses and required observations. However few deviations are observed such as the number of animals which is too low in each group (not enough dams to be evaluated when mortality occurred in the group), the period of administration which was from GD7 to 19 and the lack of data on food consumption. There are no statements concerning GLP but the study was subjected to Quality Assurance inspections.

Results

One animal in the 100 mg/kg bw/day dosage group died on GD27. At necropsy examination the doe had congestion of the lungs. Antemortem observation showed no antemortem abnormalities for the rest of the treated animals. A sacrifice in extremis in the control group and a death at the 10 mg/kg bw/day dosage level were not considered treatment-related. There were no treatment-related effects observed with respect to body weight.

Does at each treatment level incurred whole litter resorptions (ranged between 6% and 19%). Comment: *The historical control incidence of does with resorptions only, in studies performed on New Zealand White rabbits for animals delivery dates between May 1985 and February 1988 at the laboratory in question (* [redacted] *Michigan) ranged between 5% and 12%. However, the number of whole litter resorptions decreased at each successively higher dosage level and all other examined cesarean section values were comparable to values of the control group. Due to that, the effect was not considered to be substance-related.*

At the 100 mg/kg bw/day dosage level, ossification was retarded in several skeletal districts. Additionally, the number of foetuses with 27 presacral vertebrae was increased in incidence among treated does compared to the control group. This effect showed no dose-response pattern and was not as marked on a litter basis.

As it can be seen in the following table, the mean foetal body weight was reduced in the treated groups due to increase mean litter size. Therefore the retarded ossification could be due to a slight decreased in body weight.

Table 5.6.1-01: Summary of the incidence of the main foetal development variations concerning retarded ossification: number of fetuses (number of the litters)

Concentrations in mg/kg/day	0	10	25	100
Number of litters examined	12	12	10	13
Number of foetuses examined	74	93	86	96
Hyoid body unossified	1 (1)	8 (3)	10 (3)	19 (5)
Sternebra 5 and/or 6 unossified	5 (4)	9 (6)	25 (5)	21 (5)
Pubic bone unossified		3 (1)	1 (1)	10 (3)
Tail unossified		4 (2)	4 (2)	6 (4)
27 presacral vertebrae	6 (4)	28 (8)	18 (7)	31 (8)
Greater than 12 pairs of full ribs	32 (9)	48 (12)	43 (9)	53 (11)
Mean foetal body weight (g)	40.6±7.7	35.8±7.6 (-11.8%)	36.4±12.2 (-10.3%)	37.1±7.9 (-8.6%)
Mean litter size (viable pups with body weight data)	6.54	7.6	8.3	7

Comments from the former RMS Sweden

NOEL for developmental toxicity was 25 mg/kg bw/day based on retarded ossification and increased number of foetuses with 27 presacral vertebrae noted in rabbits receiving deltamethrin at 100 mg/kg bw/day. There are some deviations from OECD guideline no 414. Only one control group was used (the vehicle control group). There were no data concerning the food consumption. There are no statements concerning GLP but the study was subjected to Quality Assurance inspections and seems to be of acceptable quality.

Report: KCA 5.6.01; [redacted]; 2001; M-204103-01-1
Title: Prenatal developmental toxicity study by oral route (gavage) in rabbits Deltamethrin
Report No.: C017345
Document No.: M-204103-01-1
Guideline(s): JMAF: 4200; OECD: 414; USEPA (=EPA): OPPTS 870.3700
Guideline deviation(s): -
GLP/GEP: yes
***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity 99.1%) dissolved in corn oil was administered orally by gavage to mated female rabbits of the KBL New Zealand White strain (24 animals/dose level) at dose levels of 0 (control), 3, 10 or 32 mg/kg bw/day from day 6 to day 28 *post-coitum* inclusive. The control animals received the vehicle (corn oil) only. On day 29 *post-coitum*, the does were sacrificed, the gravid uterus was weighed, and a macroscopic post-mortem examination performed. The foetuses were removed by hysterectomy. All the foetuses were weighed and subjected to an external examination. The live foetuses were subjected to fresh dissection and detailed examination of the soft tissue, after which the carcasses were fixed and the skeletons stained and thoroughly examined (bone and cartilage).

This study follows the OECD guideline 414 revised in January 2001.

Results

Maternal data

One female of the control group died while aborting on day 22 of pregnancy, and another one was sacrificed on day 20 of pregnancy following evidence of abortion. One female of the low dosage group died just after gavage on day 7 *post-coitum*, and two females of the same dosage group were found dead on days 15 and 17 *post-coitum*, respectively. Two females of the intermediate group died just after gavage on days 22 and 27 *post-coitum*, respectively. One female of the high dosage group died just after gavage on day 28 *post-coitum*, and two females of the same dosage group were found dead on days 14 and 21 *post-coitum, respectively*. In addition, one female of the high dosage group died while aborting on day 27 of pregnancy, and another one was sacrificed on day 26 of pregnancy following evidence of abortion. However, no deaths or abortions in any group were considered to be related to the toxicity of the test substance. No clinical signs were considered to be treatment-related.

Decreased food consumption (not statistically significant, 22% decrease) and lower body weight gain (not statistically significant, 68% decrease) were noted for females of the highest dosage group when compared to control group.

No macroscopic findings were considered to be treatment-related at any dose level.

Litter data

No treatment-related effects were observed on the number of corpora lutea, the number of implantation sites, the pre- or post-implantation loss, the number of live foetuses and the foetal body weight.

Foetal examination

No treatment-related malformations or variations were noted in the foetuses of any group, following external, soft tissue and skeletal examinations.

Conclusion

NOEL for maternal toxicity was 10 mg/kg bw/day based on decreased food consumption and body weight gain noted for animals of the highest (32 mg/kg bw/day) dosage group. NOEL for developmental toxicity was 32 mg/kg bw/day.

Comments from the former RMS Sweden

The study follows OECD guideline no 414. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Results obtained in the mouse:

Report:	KCA 5.6/02; [redacted]; 1977; M-093444-01-1
Title:	RU 22974. Teratological Study in Mouse - Rat - Rabbit
Report No.:	A20256
Document No.:	M-093444-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity not specified) dissolved in sesame oil was administered by gavage to mated female Swiss CD 1 mice (24 animals/group) at dose levels of 0 sesame oil control), 0.1, 1 and 10 mg/kg bw/day during days 6-17 of gestation. Mating (accomplished naturally) was verified by detecting a copulatory plug. The day of observation was considered as day 0 of gestation. Mice were sacrificed on day 18 of gestation. Dams and foetuses were then examined.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses, running a concurrent positive control group and required observations. However few deviations are observed such as the volume of administration which is 0.4 ml/animal instead of 0.4ml/100 g of body weight, the purity of deltamethrin which is not specified, the housing conditions in the experimental room which are not mentioned. The study was not conducted under GLP, but the data seem robust enough.

Results

Mouse

Maternal deaths (considered by the author to be not treatment-related) occurred at the 1 and 10 mg/kg bw/day dosage levels (3/24 and 1/24 for the group, respectively). These animals were either autolysed or had been partly eaten by their cage-mates. No clinical signs of toxicity were observed in the surviving animals.

Maternal bodyweight gain was similar for control and treated mice.

No effects were observed on the number of implantation sites or fetal mortality. Dose-related statistically significant decreased mean fetal weight (7-9%) was observed in comparison with controls in the medium and high dosage groups (1 and 10 mg/kg bw/day). Statistically significant delayed ossification of the paws was noted in foetus of dams receiving deltamethrin at the dose levels of 0.1 mg/kg bw/day (25%), 1 mg/kg bw/day (33%) and 10 mg/kg bw/day (29%) when compared to the control group animals (14%) (the values were within normal limits for historical control animals). Statistically delayed ossification of the sternebrae was noted in foetuses of dams receiving deltamethrin at the dose level of 1 mg/kg bw/day (40%) and 10 mg/kg bw/day (37%) when compared to the control group animals (25%) (the values were within normal limits for historical control animals).

Comments from the former RMS Sweden

NOEL for maternal toxicity in mice was >10 mg/kg bw/day. NOAEL for developmental toxicity in mice was 10 mg/kg bw/day. Delayed ossification was noted in foetuses of dams receiving deltamethrin at the dose level of 0.1, 1.0 and 10 mg/kg bw/day. Additionally, decreased mean fetal weight was noted in foetuses of dams receiving deltamethrin at the dose levels of 1 and 10 mg/kg bw/day. The absence of overt maternal toxicity in the highest dosage group animals may indicate that the highest dosage level was too low.

Report:	KCA 5.2/01; [redacted] 1979; M-094154-01-1
Title:	Toxicity studies with decamethrin, a synthetic pyrethroid insecticide
Report No.:	A20968
Document No.:	M-094154-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity not specified) dissolved in corn oil was administered by gavage to CD-1 mice (30 animals/group) at doses of 0 (corn oil control), 1.0 and 10 mg/kg bw/day during days 7-16 of gestation. Day 1 of pregnancy was recorded upon demonstration of a copulatory plug. Mice were sacrificed on day 18 of gestation. Dams and foetuses were then examined.

This study mainly fulfils requirements in OECD guideline 414 revised in January 2001 with regard to study design, doses, running a concurrent positive control group and required observations. However few deviations are observed such as the purity of the test substance is not specified and the housing and feeding conditions which are not mentioned. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed). The study seems to be of acceptable quality.

No dose-related occurrences of maternal mortality were observed. Clinical signs (convulsions) were noted in animals from the middle and high dosage groups. Dose-related statistically significant reduced maternal body weight gain were noted.

No effects were observed on the number of implantation sites, fetal mortality, or fetal weights, or in the number of sternal and caudal ossification centers.

A significant ($p \leq 0.01$) dose-related increase in the occurrence of supernumerary ribs was observed, but with no dose-related effect.

Comments from the former RMS Sweden

No NOEL for maternal toxicity was determined for mice or rats due to reduced maternal body weight gain. An increased incidence of supernumerary ribs were noted in mice from all treated groups. Due to this fact, no NOEL for developmental toxicity in mice was determined. The reference is a published article (J. Env. Path, and Tox. 2:751-765, 1979) which consists of a summary of toxicity studies on deltamethrin conducted by the E.P.A, United States. No raw data were available. The study seems to follow OECD

guideline no 414 except for some minor deviations. The purity of the test substance was not specified and there were lack of data concerning housing or feeding conditions. There are no statements concerning GLP or Quality Assurance inspections (GLP was not compulsory at the time when the study was performed). The study seems to be of acceptable quality.

CA 5.7 Neurotoxicity studies

The neurodevelopmental toxicity study was conducted as confirmatory data for the EU in the frame of the last Annex I listing. However the study has not been evaluated at the EFSA level and is not part of the baseline dossier. It will be summarized in this dossier. For the previously submitted neurotoxicity studies, a copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.

In an old study performed in domestic hens, the LD_{50} was higher than 5000 mg/kg/day in corn oil and no neuropathy was observed.

In the acute rat study, deltamethrin (dissolved in corn oil) induced mortality (one male and one female), decreased body weight gain, clinical signs (gait alteration and yellow staining on the abdomen and/or urogenital area) and transient alterations of the functional observation battery (observed at the time peak effect only) at 50 mg/kg. The alterations seen in the FOB affected all six of the functional domains described by [redacted] (i.e., sensorimotor, autonomic, neuromuscular, physiological, activity and excitability). In addition, potential effects (limited to a single male and female) were observed at a dose level of 15 mg/kg (slight salivation, slightly soiled fur and slightly impaired mobility). Therefore, the NOEL for acute neurotoxicity was 5 mg/kg.

In the 90-day rat subchronic study, deltamethrin was administered via the diet to Sprague Dawley rats at concentrations of 50, 200 and 800 ppm (4, 14 and 54 mg/kg/day in males and 4, 16 and 58 mg/kg/day in females). Treatment-related effects were mainly limited at 800 ppm (54/58 mg/kg/day in both males and females, respectively) and consisted in mortality (three males and two females), functional alterations and reduced body weight and food consumption. Functional alterations seen at 800 ppm in both sexes were gait alteration, hypersensitivity to noise, impaired righting reflex, piloerection, convulsions, popcorn seizures, altered posture, increased incidences of tan matting/staining. No treatment-related neuropathological lesions were observed. In the 200 ppm group, gait alterations and hypersensitivity to noise were observed in a few animals on single occasions. In the 50 ppm group, gait alterations, hypersensitivity to noise and piloerection were each noted for single animals. On that basis the dose level of 50 ppm was considered to be the NOAEL (corresponding to achieved dosages of 4.0 mg/kg bw/day).

A Developmental neurotoxicity pilot study was conducted to verify deltamethrin exposure of the offspring during lactation (PND 10 to 16) and to determine how well Wistar rats would tolerate exposure to a dietary concentration of 250 ppm from GD 6 through day 16 of lactation. In this study, dietary concentration of 250 ppm from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats caused an increased incidence of pup loss, including cannibalization by the dam, during the first week of lactation, indicating toxicity to either the dam or offspring. In addition, deltamethrin levels were determined in brain tissue from offspring at PND 10 (34.65 ± 7.67 ppb), 14 (37.19 ± 7.47 ppb) and 16 (32.08 ± 5.02 ppb) thus indicating that the dietary mode of administration is adequately representative of early food intake by infants and potential direct exposure of children.

In the definitive Developmental Neurotoxicity (DNT) study technical grade deltamethrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 20, 80 and 200 ppm, corresponding to a mean daily intake of 0, 1.64, 6.78 and 16.1 mg deltamethrin/kg bw/day. Offsprings were subjected to evaluation using the following observations and measurements: detailed clinical observations and a functional observational battery, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60±2 days) and an ophthalmic examination. Neural tissues were collected on PND 21 and at study termination (approximately 75 days of age) for microscopic examination and morphometry.

In dams treated at 20 and 80 ppm, there were no treatment-related findings during gestation or lactation. Maternal effects (reduced body weight (6-7%) and bodyweight gain (17%), and reduced food consumption) were noted at 200 ppm (16.1 mg/kg bw/day). Effects in the offspring including reduced preweaning body weight (males: 10%, females: 9%), reduced bodyweight gain (males: 15%; females: 18%), increased incidence of vocalizations with handling in males on PND 4 and a delay in balanopreputial separation (45.1 days vs. 43.5 days for controls) were noted at 200 ppm (16.1 mg/kg bw/day).

The maternal NOEL was 80 ppm (6.78 mg/kg bw/day) based on reduced bodyweight gain (>10%) noted in dams at the concentration of 200 ppm. The offspring NOEL was 80 ppm based on reduced bodyweight gain (>10%), increased incidence of vocalizations with handling (males only) and delayed balanopreputial separation noted in offsprings at the concentration of 200 ppm. The delay in balanopreputial separation could be explained by the pup decreased body weight. Therefore this study demonstrates that deltamethrin does not raise any concern for developmental neurotoxicity. It is also the position of the PPR Panel in their scientific opinion on potential developmental neurotoxicity of deltamethrin adopted in December 2008. The PPR Panel concluded that deltamethrin has been adequately tested for developmental neurotoxicity and that the available data do not indicate that deltamethrin is a developmental neurotoxic agent.

Table 5.7-01: Summary of neurotoxicity studies of deltamethrin (new studies not yet submitted highlighted in black and bold – studies in the baseline dossier in gray)

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
Acute hen neurotoxicity [redacted] 1978 M-093518-01-1		3000		5000	One mortality at 5000 mg /kg, no ataxia and no neuropathy
Acute rat neurotoxicity [redacted], 1998 M-152563-01-1 0, 5, 15, 50 mg/kg		5		15	Neurological signs (salivation, impaired mobility)

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Adverse effects at LOAEL
	ppm	mg/kg/d	ppm	mg/kg/d	
90-day rat neurotoxicity [redacted], 1998 M-152562-01-1 0, 50, 200, 800 ppm	200	14/16	800	54/58 in M/F	Gait alteration, convulsions, tremors and altered FOB No neuropathological findings
Rat pilot DNT study [redacted] **2006 M-276949-01-1 250 ppm**	<250		250		Pup loss including cannibalization by the dams
Rat DNT study [redacted] **2006 M-270180-03-1 0, 20, 80, 200 ppm**	Dams: 80 Offspring: 80	6.78 6.78	200 200	16.1 16.1	↓ BWG in dams and offspring , ↑vocalizations with handling (males only) and delayed balanopreputial separation due to decreased BW

CA 5.7.1 Neurotoxicity studies in rodents

Report:	KCA 5.7/01; [redacted]; 1978; M-093518-01-1
Title:	Ru 22974 (decamethrine) LD50 determination and assessment of neurotoxicity in the domestic hen.
Report No.:	A20307
Document No.:	M-093518-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

***Annex data point due to first Annex I listing**

Experimental design

The study was designed to determine the LD$_{50}$ to domestic hens and to assess the neurotoxic hazard following a single orally dosing by gavage with RU 22974 (deltamethrin) (purity not specified). At the higher dose levels the required dose volumes were given as a number of split doses administered over a 6-hour period. The study was divided into the following sections:

(a) Determination of the LD$_{50}$ value of RU 22974 to the domestic hen using corn oil as the vehicle with the compound in suspension (10 animals/group). Dose levels were 800, 1200, 1600, 2000, 3000 and 5000 mg/kg bw.

(b) Determination of the LD$_{50}$ value of RU 22974 to the domestic hen using sesame oil as the vehicle with the compound in solution (8-10 animals/group). Dose levels were 1000 and 2500 mg/kg bw.

(c) Assessment of ataxia in the domestic hen following dosing with RU 22974. The vehicle used was corn oil with the compound in suspension (10 animals/group). Dose levels were 0, 500, 1250 and 5000 mg/kg bw. A positive control group received tri-orthocresyl phosphate (TOCP) at 500 mg/kg bw.

(d) Assessment of ataxia in the domestic hen following dosing with RU 22974. The vehicle used was sesame oil with the compound in solution (10 animals/group). Dose levels were 0 and 1000 mg/kg bw.

The birds used in the determination of the LD_{50} value were observed for 14 days. Birds used to assess neurotoxic effects were observed for 21 days and then the sciatic nerve and spinal cord of all animals were examined histopathologically.

Results

LD_{50} in domestic hens was greater than 5000 mg/kg bw (highest dose level tested) when a corn oil-solution of deltamethrin was used. LD_{50} in domestic hens was greater than 2500 mg/kg bw (highest dose level tested) when a sesame oil-solution of deltamethrin was used. There was an increase in toxicity following dosing with the compound in solution in sesame oil compared with dosing as a suspension in com oil. No adverse clinical signs were noted that could be related to dosing with RU 22974. No signs of ataxia were observed in any of the birds dosed with RU 22974. All birds in the positive control group showed signs of ataxia ranging from moderate to severe, as expected.

A slight treatment-related decrease in body weight gain was observed following dosing with RU 22974 at the concentrations of 2500 (when sesame oil was used as the vehicle) and 5000 mg/kg bw (when corn oil was used as the vehicle).

No treatment-related macroscopic or histological changes were found in the sciatic nerve or spinal cord in birds dosed with RU 22974. Degenerative changes seen in the spinal cord and sciatic nerve were found in animals in the positive control group.

Comments from the former RMS Sweden

The results of this study pointed out the importance of the choice of vehicle. A suspension of deltamethrin in corn oil or a solution of deltamethrin in sesame oil seems to be poorly absorbed in hens. Deltamethrin, did not produce any detectable signs of neurotoxicity in domestic hens under the conditions used in this study. NOEL for domestic hens when sesame oil was used as the vehicle with the compound in solution was >1000 mg/kg bw (reduced body weight gain were noted at the concentration of 2500 mg/kg bw in birds used in the study designed for determination of LD_{50}). NOEL for domestic hens when corn oil was used as the vehicle with the compound in solution was 1250 mg/kg bw based on reduced body weight gain noted in hens receiving the test substance at 5000 mg/kg bw. A serious shortcoming is that the test substance used in this study was a solution or a suspension of deltamethrin, and not undiluted deltamethrin which is preferable. This fact restricts the sensitivity of the test. No OECD guidelines exist for this type of study although the study follows OECD guideline no 418 (for acute delayed neurotoxicity of organophosphorus substances) in most aspects except for the fact that the animals were not redosed and observed for another 21 days for delayed neurotoxicity, and no histopathologically examination of medulla oblongata was made. There are no statements concerning GLP or Quality Assurance inspections. The study is not of acceptable quality for assessment of neurotoxicity of deltamethrin due to the choice of suspended/soluted test substance instead of undiluted deltamethrin.

Report:	KCA 5.7/02; [redacted]; 1998; M-152563-01-1
Title:	An acute neurotoxicity study of deltamethrin in rats.
Report No.:	A74318
Document No.:	M-152563-01-1
Guideline(s):	USEPA (=EPA): 81-8-SS, (1991)
Guideline deviation(s):	--
GLP/GEP:	**yes**

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity 99.2%) was dissolved in corn oil and administered orally by gavage as a single dose to non fasted rats of [redacted] BR strain (12 animals/sex/group) at dose levels of 5, 15 and 50 mg/kg bw. The controls (12 animals/sex) received corn oil only. Experimental parameters recorded for all animals included viability, clinical signs, body weights and Functional Observational Battery and Locomotor Activity evaluations. The functional tests were recorded during pre-test, at the time of peak effect and on study days 7 and 14 for all animals. The functional tests included sensory reactivity to stimuli of different modalities, assessment of limb grip strength and assessment of motor activity. All surviving animals were euthanized on study day 15 and perfused *in situ*. Brain weight (excluding olfactory bulbs) and brain dimensions were recorded for each of these animals. A neuropathological evaluation was performed on five animals/sex in the control and 50 mg/kg bw groups.

The study complies with the OECD guideline 424, published in July 1997.

Results

Deaths occurred on the day of test article administration in one male and one female animal receiving deltamethrin at the dose level of 50 mg/kg bw. Upon macroscopic examination, pale lungs were observed in the male, and mottled lungs and an ocular opacity were noted in the female. Gait alterations, yellow staining on the abdomen and/or urogenital area, tan staining around the mouth and/or on the forelimbs were noted in animals receiving deltamethrin at the dose level of 50 mg/kg bw.

Statistically significant decreased mean body weight gain was noted (for study days 0 to 7) for males receiving deltamethrin at the dose level of 50 mg/kg bw.

When the Functional Observational battery was performed at the time of peak effect (approximately 3-hours post-dosing) the following signs of toxicity were apparent for animals receiving deltamethrin at the dose level of 50 mg/kg bw: altered posture, convulsions (clonic and tonic), tremors, alterations in biting and palpebral closure, alterations in ease of removal from the cage, reduced ease of handling, lacrimation, salivation, slight soiled fur appearance, chromodacryorrhea, increased time to first step, impaired mobility and gait, decreased arousal, bizarre and/or stereotypic behaviour, decreased rearing, grooming, urination, absent approach response, absent touch response, absent startle and tail pinch responses, absent olfactory orientation, altered forelimb and hindlimb extension, altered air righting reflex, reduced forelimb and hindlimb grip strength, impaired rotarod performance and altered hindlimb footsplay (males only). Additionally, increased group mean catalepsy values and decreased group mean body temperatures were noted for animals receiving deltamethrin at the dose level of 50 mg/kg bw. Slight salivation, slightly soiled fur and slightly impaired mobility were noted in some

animals receiving deltamethrin at the dose level of 15 mg/kg bw/day when the Functional Observational battery was performed at the time of peak-effect (approximately 3-hours post-dosing).

Increased mean ambulatory and total motor activity counts were apparent for males receiving deltamethrin at the dose level of 50 mg/kg bw when the Locomotor Activity evaluation was performed on study day 0.

No treatment-related effects were apparent in brain weights or brain dimensions for perfused animals. One male animal receiving deltamethrin at the dose level of 50 mg/kg bw had digestion chambers in the sciatic nerve (with axonal degeneration) and tibial nerve. One female animal receiving deltamethrin at the dose level of 50 mg/kg bw had digestion chambers in the sciatic and peroneal nerves. The effects noted were recorded as minimal to mild. The differences from the control group values were not statistically significant. Further on, nerve fiber degeneration, characterized by digestion chambers may spontaneous occur in control animals. Therefore, the increased incidence of digestion chambers in peripheral nerves (see Table 5.7.1-01) and axonal degereration noted in animals receiving deltamethrin at the dose level of 50 mg/kg bw was by the author considered spontaneous and unrelated to the administration of the test substance. Historical control incidence of digestion chambers in peripheral nerves in studies performed on rats for animals delivery dates between 1991 and 1992 for the laboratory in question ranged between 0 and 33.3% for the sciatic nerve, 0 and 16.7% for the tibial nerve and was 0% for the peroneal nerve.

Table 5.7.1-01: Incidence of degeneration (digestion chambers) in peripheral nerves of a small no of animals (5 animals/sex/group).

Dose level (mg/kg bw)	Sciatic nerve	Tibial nerve	Peroneal nerve
0	none	none	none
50	20% (m) and 20% (f)	20% (m) and none (f)	none (m) and 20% (f)

Comments from the former RMS Sweden

The NOEL for male and female rats was 5 mg/kg bw based on functional alterations (slight salivation, slightly soiled fur and slightly impaired mobility) noted in animals that received deltamethrin at a dose level of 15 mg/kg bw. Additionally, deaths, clinical signs (gait alterations, yellow staining on the abdomen and/or urogenital area, tan staining around the mouth and/or on the forelimbs), decreased mean body weight gain (males only), functional alterations (altered posture, convulsions (clonic and tonic), tremors, alterations in biting and palpebral closure, alterations in ease of removal from the cage, reduced ease of handling, lacrimation, chromodacryorrhea, increased time to first step, impaired mobility and gait, decreased arousal, bizarre and/or stereotypic behaviour, decreased rearing, grooming, urination, absent approach response, absent touch response, absent startle and tail pinch responses, absent olfactory orientation, altered forelimb and hindlimb extension, altered air righting reflex, reduced forelimb and hindlimb grip strength, impaired rotarod performance, altered hindlimb footsplay (males only), increased mean ambulatory and total motor activity counts (males only)) and degeneration in peripheral nerves were noted in animals receiving deltamethrin at the dose level of 50 mg/kg bw. The study follows OECD guideline no 424 except for some deviations. No data concerning food consumption was given. No neuropathological evaluation was performed on samples from nervous tissues from the intermediate and low dose groups. This fact severely restricts the sensitivity of the study. According to the guideline no 424 a stepwise examination of tissue samples is recommended in which sections from the high dose group are first compared with those of the control

group. If any neuropathological alterations are observed in the high dose group, a second examination should be performed on all regions of the nervous system showing these alterations. At the second examination samples from nervous tissues from the intermediate and low dose groups should be examined. Deltamethrin is a substance expected to cause signs of nervous system toxicity. Increased incidence of digestion chambers in peripheral nerves with or without axonal degeneration was noted in two animals receiving deltamethrin at the dose level of 50 mg/kg bw compared to the control group (0%). Although the differences from the control group values were not considered statistically significant (note: statistical test was performed on a small group of animals), further neuropathological evaluation should according to the guideline be performed on samples from nervous tissues from the intermediate and low dose groups. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study is not of acceptable quality due to the absence of a further neuropathological examination.

A comment from the notifier is that the finding of "digestion chambers" is more common than above historical control data suggest. In addition the notifier has provided further historical (1992-present) control data from subchronic (three months duration) neurotoxicity studies conducted in [redacted] and [redacted]BR rats which show that digestion chambers in peripheral nerves have more often been found as spontaneous lesions in the rat (incidence digestion chambers: 40% sciatic nerve, 20% peroneal nerve). When taken into consideration and that results from chronic toxicity and subchronic toxicity studies for deltamethrin gave no indication of treatment-related neuropathological changes in the rats the notifier concludes that additional neuropathological evaluation is not necessary ([redacted], 2001, M-240464-01-1).

Report:	KCA 5.7/04; [redacted]; 1998; M-152562-01-1
Title:	A subchronic (13-week) neurotoxicity study of deltamethrin in rats.
Report No.:	A74317
Document No.:	M-152562-01-1
Guideline(s):	USEPA (=EPA): 82-7, (1982)
Guideline deviation(s):	--
GLP/GEP:	yes

***Annex data point due to first Annex I listing**

Experimental design

Deltamethrin (purity 99.2%) was administered in the diet to rats of the [redacted]BR strain (10 animals/sex/group) at concentrations of 50, 200 and 800 ppm for a period of 13 weeks. The concentrations corresponded to a dose rate of 4, 14 and 54 mg/kg bw/day for males, respectively, and 4, 16 and 58 mg/kg bw/day for females, respectively. The controls (10 animals/sex) received the diet only. Experimental parameters recorded for all animals included viability, clinical signs, body weights, food consumption and Functional Observational Battery and Motor Activity evaluations. The functional tests were recorded during pre-test and then on study weeks 3, 7 and 12 for all animals. The Functional Observational Battery and Motor Activity evaluations included sensory reactivity to stimuli of different modalities, assessment of limb grip strength and assessment of motor activity. Brain weight (excluding olfactory bulbs) and brain dimensions were recorded for each animals. In addition, *in situ* tissue perfusion was performed on each animal. A neuropathological evaluation was performed on five animals/sex in the control and 800 ppm groups.

The study complies with the OECD guideline 424, published in July 1997.

Results

Treatment-related deaths occurred in animals receiving deltamethrin at a concentration of 800 ppm (three male and two female rats died). In addition, another female in the 800 ppm group was euthanized *in extremis* due to mechanical trauma. Hypersensitivity to noise and gait alterations (rocking, lurching or swaying, walking with hindlimbs splayed, walking on tiptoes and/or writhing) were noted in the 200 and 800 ppm group males and females. Other observed behaviour/CNS signs in the 800 ppm group males and females included impaired righting reflex, piloerection, convulsions, popcorn seizures and altered posture. Additionally, increased incidences of tan matting/staining were noted in the 800 ppm group males and females.

In the 200 ppm group, gait alterations were observed for four males and two females and hypersensitivity to noise was observed for one male and two females. In the 50 ppm group, gait alteration was noted in one female, hypersensitivity to noise in another female and piloerection in one male. With the exception of one male from the 200 ppm group, these findings were seen on a single occasion during the mid-point of the study (approximately 6 weeks after the start of treatment on August 26 for all animals and August 27 and September 02 for one male from the 200 ppm group) by an alternate observer and were not noted on the following day when the usual observer examined these animals. Additionally, none of these findings were apparent in these groups at the Functional Observational Battery. Therefore, no relationship to treatment was considered by the applicant. All animals from the 800 ppm group exhibited neurological signs usually 10 days after the start of treatment and these signs were observed until the animals were found dead or sacrificed at the end of the study. The convulsions and popcorn seizures were only observed in animals which died during the study except for one female.

Statistically significant decreased mean body weights were noted in the 800 ppm group males and females. The lower mean body weights were primarily due to low mean body weight gains and/or mean body weight losses that occurred during the first three weeks of the study. Statistically significant reduced mean food consumption was noted in the 800 ppm group males and females throughout the study.

When the Functional Observational battery was performed on study weeks 3, 7 and 12 following signs of toxicity were apparent for animals that received deltamethrin at a concentration of 800 ppm: piloerection (males only at study week 3), slightly soiled fur (males only at study week 3), impaired mobility and gait, bizarre/stereotypic behaviour, altered air righting reflex, altered hindlimb extensor strength, reduced forelimb grip strength (males only at study weeks 3 and 7), reduced hindlimb grip strength (males only at study week 3).

No treatment-related effects were apparent between treated and control group animals when the Locomotor Activity evaluations were performed.

No remarkable differences between the treated and control group animals were observed in brain weights or dimensions. No treatment-related neuropathological lesions were observed at the microscopic examination of perfused tissues.

Comments from the former RMS Sweden

The NOEL for male and female rats was 50 ppm (4 mg/kg bw/day for males and females) based on clinical signs (hypersensitivity to noise and gait alterations) noted in animals that received deltamethrin at a concentration of 200 ppm. Additionally, deaths, clinical signs (impaired righting reflex, piloerection, convulsions, popcorn seizures, altered posture, increased incidences of tan matting/staining), reduced food consumption, decreased mean body weights and functional alterations (impaired gait, impaired mobility, bizarre/stereotypic behaviour, altered air righting reflex, altered hindlimb extensor strength, reduced hindlimb grip strength (males only), reduced forelimb grip strength (males only) and slightly soiled fur) were noted for animals that received deltamethrin at a concentration of 800 ppm. The study follows OECD guideline no 424. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Conclusion from the applicant:

The NOAEL was considered to be 200 ppm (14/16 mg/kg/day in males and females, respectively) based on mortality, clinical signs and alteration of FOB seen at 800 ppm.

The neurodevelopmental toxicity study was conducted as confirmatory data for the EU in the frame of the last Annex I listing.

Report:	KCA 5.7.1/01; [redacted]; 2006; M-276949-01-1
Title:	A pilot study to verify the exposure of offspring during lactation to technical grade Deltamethrin administrated via the diet to Wista rats
Report No.:	04-P72-VX
Document No.:	M-276949-01-1
Guideline(s):	U.S. EPA, OPPTS 870.6300 OECD draft TG 426 (September 2003)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive Summary

Technical grade deltamethrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 16 to mated female Wistar rats at a nominal concentration of 250 ppm, with adjustments during lactation to maintain a more consistent dosage throughout exposure. The test diet was provided for *ad libitum* consumption throughout the study. Concentration in the diet, as well as the homogeneity and stability of deltamethrin in the dietary ration, was confirmed. On postnatal day (PND) 4, litters with a minimum of seven pups, including at least two per sex, were culled to yield, as closely as possible, four males and four females. Dams and/or pups were subjected to evaluation using the following observations and measurements - detailed clinical observations, body weight and food consumption (dams; GD 6-13, 13-20 and LD 0-7 and 7-14). In addition, whole brain was collected from the first three male and three female offspring in each litter to assay for deltamethrin on PND 10, 14 and 16 (one/sex/litter at each age).

Treatment-related effects attributed to exposure to deltamethrin were as follows:

250 ppm **-** Increased incidence of pup loss, including cannibalization by the dam, during the first week of lactation.

Table 5.7.1-02: Mean brain tissue analysis was as follows:

PND 10		PND 14		PND 16	
	Level (ppb)		Level (ppb)		Level (ppb)
Mean ± S.D.	34.65 ± 7.67	Mean ± S.D	37.19 ± 7.47	Mean ± S.D	32.08 ± 5.02

These results clearly establish that the offspring were exposed to deltamethrin during lactation. This outcome supports dietary administration in the DNT study at dietary concentrations of 0, 20, 80 and 200 ppm.

I. Materials and Methods

A. Material

1. Test Material: Deltamethrin technical
Description: White powder
Lot/Batch: 2350014
Purity: 98.8%
CAS: 52918-63-5
Stability of test compound: Stable at 3 and 1000 ppm in the diet at the room temperature for 7 days or after 42 days in the freezer

2. Vehicle and /or positive control: None

3. Test animals:
Species: Rat, Wistar Han([redacted]BR)
Age: 12 (females) or 15 (males) weeks of age at co-housing
Weight: at ± 20% weight determination for the females
Males had no specified weight requirements.
Source: [redacted]
Acclimation period: At least 6 days
Diet: [redacted] Certified Rodent Diet Meal [redacted] provided for *ad libitum* consumption during the acclimation period and throughout the study.
Water: Tap water ([redacted] Missouri [redacted]), *ad libitum*
Housing: Suspended stainless steel cages; individually, except during co-habitation (one male with one female) with deotized cage board in the bedding tray; individually (or with litters) in plastic cages with corn cob bedding during gestation and lactation. Each cage contained a feeder and source of water (pressure-activated water lixits and/or water bottles).

Environmental conditions:	Temperature:	22 ± 4 °C
	Humidity:	50 ± 20% (relative)
	Air changes:	Minimum daily average of 10 air changes per hour
	Photoperiod:	Alternating 12-hour light and dark cycles

B. Study Design

1. In life dates – June 8, 2004 to July 22, 2004

2. Animal assignment and treatment

Upon arrival, animals were randomly assigned an identification number as they were arbitrarily selected and removed from the shipping crates and placed into individual cages. Random assignment of males utilized applications from SAS [2]. Following at least six days of acclimation, P-generation females were weighed and those with body weights more or less than 20% of the mean weight were rejected. The requisite number of females was assigned to the one dose group. Females not placed on study were sacrificed without necropsy. However, alternative uses for those animals were explored prior to their sacrifice. P-generation males served only as breeders. As such, they had no specified weight requirements and were arbitrarily selected for co-housing with females.

Animal mating

Mating was accomplished by co-housing one female with one male for up to five consecutive days. Cohabitation on the first day of the mating phase began by placing mating pairs together in suspended stainless steel gang cages. Each morning during the co-habitation phase, the dams and cages were examined for a vaginal plug and vaginal smears were taken and examined for sperm. The day on which insemination was evident was designated day 0 of gestation (GD 0) for that female. On GD 0, the females that were presumed to be pregnant were housed individually in a plastic nesting cage. Females not found sperm positive within the five-day limit were sacrificed without a necropsy performed.

P-generation males and females were identified by cage card and tail mark (males) or tail tattoo (females). F_1-generation animals that were born alive were identified by tattoo; pups found dead were identified with a marking pen.

The rationale for dose selection was based on the results of a two-generation reproduction toxicology study (M-149348-01-1) In that study, the highest dietary level (320 ppm) produced marked evidence of toxicity (e.g., decreased body weight gain beginning on day 8 of exposure, one death of a parental-generation female (prior to mating), and clinical signs (during lactation)). These results indicate that 320 ppm was too toxic for use in a developmental neurotoxicity (DNT) study, so 250 ppm was selected as the highest dietary level for the DNT study. Since 250 ppm was planned for use in the DNT study, it was appropriate for the present study.

3. Test substance preparation and analysis

The test substance was incorporated into the diet to provide the required dietary concentrations of 250 ppm. Concentrations of the test substance in the diet were measured by LC-MS/MS, for each week of

treatment. Formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet ([redacted]Certified Rodent Diet Meal [redacted]) and stored at freezer (average temperature -22 ± 4 °C) conditions. Dietary concentrations were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant dosage (mg/kg/day) throughout the period of exposure and to compensate for the increase in food consumption by dams during lactation. Based on an average feed consumption of 86 g/kg body weight/day during gestation for three other studies, the dietary level during gestation was 250 ppm and was then reduced during weeks 1-3 of lactation by factors of 1.9, 2.3 and 2.8, respectively, to adjust for average feed consumption of 160, 199 and 240 g feed/kg body weight/day, respectively. The treated feed was provided for consumption beginning on GD 6 and continuing through lactation day 16. A sample of each batch of feed mixed was taken and retained in the freezer until study completion and the analytical data deemed satisfactory.

Homogeneity and Stability Analysis:
The homogeneity and stability of the test substance in rodent feed was verified at dietary concentrations of 3 and 1000 ppm and were determined to be homogeneous and stable for seven days at room temperature (average temperature 22 ± 4 °C) and 42 days at freezer conditions (average temperature -22 ± 4 °C).

Concentration Analysis:
The concentration of the test substance in the ration was verified for each week of treatment.

Test substance analysis in target tissues:
Brains were collected from the first three male and three female (as available) offspring in each litter on PND 10, 14 and 16 (one/sex/litter at each age) to measure the concentration of the test substance in this target tissue. The remaining pups (~1/sex/litter) were sacrificed on PND 16 and discarded without a routine necropsy examination or collection of tissues.

C. Methods

1. In-life observations

a. Maternal animals:

1) Clinical Observations

Following acclimation and continuing until animals were removed from the study, P-generation males and females were observed (cage-side) for clinical signs at least once daily during the regular work week and on weekends and holidays. These observations were sufficient to characterize mortality, moribundity, behavioral changes, and overt toxicity by viewing the animal in its cage. In the event that a possible clinical sign was observed during the cage-side evaluation, the animal was removed from the cage for a more detailed examination.

2) Detailed Observations

A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through LD 16. These observations were performed by an individual who was aware of the animal's dosage group assignment.

3) Body Weight and Food Consumption

Body weight and food consumption were measured once weekly during gestation and lactation, as follows: GD 6–13, GD 13–20, LD 0–7, LD 7-14. In addition, dams were weighed on GD 0 and LD 4. Fresh feed and clean feeders were provided weekly.

4) Delivery and Culling

Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated LD 0 for the dam and PND 0 for the pups. Litter size (the number of pups delivered) and pup "status" at birth were recorded for each litter. Dams that delivered fewer than two pups per sex or litter size of fewer than seven pups by PND 4 were sacrificed (along with their litter) without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. Adjustments of litters were made by random selection of the pups, using plastic chips with numbers that were drawn at random from a container. When the number of male or female pups was less than four, a partial adjustment was made (e.g., three females and five males). If there were more than 6 acceptable litters for any dietary level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by carbon dioxide (CO_2) asphyxiation and decapitation, respectively.

5) Moribund Animals and Animals Found Dead

Parental-generation males and females that were found moribund while on study were sacrificed by CO2 asphyxiation. Parental-generation males and females that were found dead or moribund will not undergo a necropsy examination and were disposed of without routine collection of tissues.

6) Termination

Males: Following cohabitation, males were sacrificed by CO_2 asphyxiation and discarded (with the exception of selected animals that were used for training or other studies).
Females: Dams were sacrificed on LD 16, by CO_2 asphyxiation, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were sacrificed on GD 24 without necropsy examination.

b. Offspring

As soon as possible following parturition, the gender was determined and pups were tattooed and weighed.

1) Litter Observations

Gross Observations. All pups were observed (cage-side) for clinical signs at least once daily (p.m.). These observations were sufficient to characterize mortality, moribundity, neurobehavioral changes, and overt toxicity by viewing the animal in its cage. On weekends and holidays, these observations were most likely performed at the same time as the more detailed examination for clinical signs.
Detailed Observations. All offspring were subjected to detailed observations for clinical signs once daily (a.m.) during the preweaning period. These observations were performed by an individual who

was aware of assignments to dosage group. This examination included gross observations, as well as observation with handling, and included but not limited to changes in the skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic and central nervous systems, somatomotor activity and behavior.

2) Body Weight

Surviving pups were weighed on PND 0, 4, 10, and 16

3) Tissue assay for deltamethrin

At 10, 14 and 16 days of age, the whole brain was collected from one male and one female (as available) from each litter (approximately 4/sex/age, representing 4 litters) and stored in a freezer (minimum -20°C) until analysis. The first male and female, in order, were selected to provide the sample as the representative of the litter at each age. Immediately prior to collection of tissues, animals were sacrificed by decapitation (PND 10) or injection of Fatal Plus (Vortech Pharmaceuticals, Dearborn, MI) (PND 14 and 16).

2. Postmortem observations:

A necropsy was not routinely required and gross lesions were not routinely collected for microscopic examination. At study termination, animals were sacrificed by CO2 asphyxiation and discarded.

F1 generation animals that were found moribund or dead while on study were sacrificed without a routine gross necropsy examination or collection of tissues.

The pups that were selected for culling were sacrificed by decapitation and discarded without necropsy.

C. Statistical Analysis

Data were not subjected to statistical analysis.

II. Results and discussion

The results of this study demonstrated an increased incidence of pup loss, including cannibalization by the dam, during the first week of lactation, indicating excessive toxicity to either the dam or offspring. The results from brain tissue analysis (below) also clearly establish that the offspring were exposed to deltamethrin during lactation. This outcome supports dietary administration in the DNT study.

Table 5.7.1-03: Concentration (ppb) of deltamethrin in brain tissue from the offspring of lactating females treated via the diet at 250 ppm

PND 10		PND 14		PND 16	
Pup Nb / sex	Level (ppb)	Pup Nb / sex	Level (ppb)	Pup Nb / sex	Level (ppb)
008-01 M	22.29	008-02 M	53.01	008-03 M	33.01
008-07 F	36.55	008-04 F	36.89	008-09 F	38.40
041-01 M	46.88	041-02 M	38.54	041-03 M	33.95
041-05 F	40.66	041-06 F	38.66	041-07 F	37.47
052-01 M	28.42	052-03 M	31.11	052-04 M	25.70

PND 10		PND 14		PND 16	
Pup Nb / sex	Level (ppb)	Pup Nb / sex	Level (ppb)	Pup Nb / sex	Level (ppb)
052-10 F	29.74	052-11 F	27.40	052-12 F	24.43
001-02 M	37.11	001-03 M	35.76	001-05 M	30.44
001-09 F	35.55	001-11 F	36.12	001-12 F	33.26
Mean ± S.D.	34.65 ± 7.67	Mean ± S.D.	37.19 ± 7.47	Mean ± S.D.	32.08 ± 5.02

Based on these results, the dietary levels selected for the main study were 0, 20, 80 and 200 ppm, with adjustments during lactation to maintain a consistent dosage throughout the period of exposure.

III. Conclusions

These results clearly establish that the offspring were exposed to deltamethrin during lactation. This outcome supports dietary administration in the DNT study at dietary concentrations of 0, 20, 80 and 200 ppm.

Report: KCA 7.1/02; [redacted]; 2014; M-270180-03-1

Title: A developmental neurotoxicity screening study with technical grade deltamethrin in Wistar rats

Report No.: 201469-2
Document No.: M-270180-03-1
Guideline(s): US EPA OPPTS 870.6300; OECD draft TG 426 (September 2003); Health Canada PMRA DACO No. 4.5.14

Guideline deviation(s): not specified
GLP/GEP: **yes**

Executive Summary

The principal objective of this developmental neurotoxicity study was to investigate the potential for technical grade deltamethrin to produce functional and morphological effects on the nervous system of offspring from oral (dietary) exposure during pregnancy and lactation.

Technical-grade deltamethrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 20, 80 and 200 ppm, with adjustments during lactation to maintain a more consistent dosage throughout exposure. All test diets (including control) were provided for *ad libitum* consumption throughout the study, except during neurobehavioral testing. Concentration in the diet, as well as the homogeneity and stability of deltamethrin in the dietary ration, was confirmed. On postnatal day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 20 litters per level, were subjected to evaluation using the following observations and measurements - detailed clinical observations and a functional observational battery, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60±2 days) and an ophthalmic examination. Neural tissues were collected from 10/sex/dietary level (representing

approximately 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.
The mean daily intake of the test substance (mg deltamethrin/kg body wt/day) based on the average dietary consumption for the last two weeks of gestation and three weeks of lactation at nominal dietary concentrations of 20, 80 or 200 ppm, respectively, was 0, 1.64, 6.78 and 16.1 mg/kg/day. Treatment-related effects attributed to exposure to deltamethrin were as follows:

Maternal

20 ppm - There were no treatment-related findings during gestation or lactation.
80 ppm - There were no treatment-related findings during gestation or lactation.
200 ppm - Body weight (6-7%) and body weight gain (17%) were significantly decreased from GD 13 through GD 20 and body weight was significantly reduced (6-8%) from LD 0 through LD 7. Food consumption was significantly decreased on GD 6-13 (17%) and on LD 0-7 (9%). Thus, the maternal LOAEL is 16.1 mg/kg bw/day, based on decreased body weight, weight gain and food consumption during gestation and decreased body weight and food consumption during lactation. The maternal NOAEL is 6.78 mg/kg bw/day.

Offspring

20 ppm - There were no treatment-related findings.
80 ppm - There were no treatment-related findings.
200 ppm - Significantly reduced pre-weaning body weight (maximum 10%) and weight gain (maximum 18%) that began on PND 4 and persisted through four weeks after weaning in males (maximum 8%) and through one week after weaning in females (7%). Increased incidence of vocalizations with handling in males on PND 4. In addition, there was a statistically-significant delay in balanopreputial separation, compared to concurrent controls (45.1 days vs. 43.5 days for controls) due to decreased body weight at the high dose.
Thus, the offspring LOAEL is 16.1 mg/kg bw/day, based on delayed balanopreputial separation, reduced body weight and weight gain before weaning for both sexes, with recovery after weaning. The offspring NOAEL is 6.78 mg/kg bw/day.

I. Materials and Methods

A. Material

1. Test Material: Deltamethrin
Description: White solid
Lot/Batch: 2350014
Purity: 98.8%
CAS: 52918-63-5
Stability of test compound: Stable at 3 and 1000 ppm in the diet at the room temperature for 7 days or after 42 days in the freezer

2. Vehicle and /or positive control: None

3. Test animals:

Species:	Rat, Wister HAN [redacted]
Age:	At least 12 (females) or 14 (males) weeks of age at co-housing
Weight (at ± 20% weight determination):	186.4–223.4 g range for females placed on study; Males had no specified weight requirements.
Source:	[redacted]
Acclimation period:	At least 6 days
Diet:	[redacted] Certified Rodent Diet Meal [redacted] provided for *ad libitum* consumption during the acclimation period and throughout the study, except during neurobehavioral testing.
Water:	Tap water ([redacted] Missouri [redacted]), *ad libitum* except during neurobehavioral testing
Housing:	Suspended stainless steel cages; individually, except during co-habitation (one male with one female) with deotized cage board in the bedding tray; individually (or with litters) in plastic cages with corn cob bedding during gestation and lactation. Each cage contained a feeder and source of water (pressure-activated water nipples).
Environmental conditions:	Temperature: 22 ± 4 °C Humidity: 50 ± 20% (relative) Air changes: Minimum daily average of 10 air changes per hour Photoperiod: Alternating 12-hour light and dark cycles (7 am to 7 pm or 6 am - 6 pm); lights toggled off during ophthalmic examinations

B. Study Design

1. Experimental phase: – September 7, 2004 to February 4, 2005

2. Animal assignment and treatment

Upon receipt, P-generation females were examined and those considered acceptable were placed into individual cages and acclimated to their ambient laboratory conditions for at least six days prior to being placed on study. For the holding period, animal care personnel observed the animals at least once daily for moribundity and mortality. All planned or unplanned activities associated with either the animals or their rooms, as well as changes in the status of either the animals or their room, were documented. With completion of the acclimation period, a veterinarian reviewed the status of the animals prior to their release for study. Dams were assigned to detailed observational testing as shown in the Study Design Table.

P-generation females were weighed assigned to the control or an exposure group in sequence, as they were determined to be inseminated. This approach was used to ensure unbiased assignment of the animals to dose groups and an approximately equal number of litters from each dose group available for testing on a given day. Females not placed on study were sacrificed without necropsy. Animals were assigned an identification number that specifies the sex, dietary level, cage number, and

identifies it with the study. P-generation males served only as breeders. As such, they had no specific weight requirements and were arbitrarily selected for co-housing with females.

P-generation males and females were identified by cage card and tail mark (males) or tail tattoo (females). F_1-generation animals that were born alive were identified by tattoo; pups found dead were identified with a marking pen.

Offspring were assigned to testing subgroups at the time of litter standardization on PND 4. An animal allocation program written in SAS was used to assign offspring to one of the following four sets (designated A–D) for assessment at each age. One male and/or female per litter [approximately 16 (minimum 10)/sex/dietary level, representing at least 20 litters per level]: motor activity (Set A), auditory startle (Set B), passive avoidance, water maze, and detailed observational battery (Set C). On PND 21, the whole brain was collected from a separate group of randomly selected offspring (Set D; 10/sex/dietary level; representing 20 litters per level) for micropathologic examination and morphometric analysis. The remaining pups assigned to Set D (~10/sex/dietary level) were reserved for use as replacement animals or otherwise sacrificed on PND 21 without necropsy examination.

At approximately 50–60 days of age, randomly selected animals (a minimum of 10/sex/dietary level, representing at least 20 litters per level) from Sets A, B, and C were subjected to an ophthalmologic examination. At termination on PND 75 (±5 days), these animals were anesthetized and sacrificed by perfusion, with neural and muscle tissues collected for micropathologic examination. At termination on PND 75 (±5 days), brains were collected from additional randomly selected animals (10/sex/dietary group; representing 20 litters per level). These brains were weighed (fresh tissue weight) and then discarded.

The remaining animals assigned to sets A–C were sacrificed without routine gross necropsy examination or collection of tissues.

Table 5.7.1-04: Study design and animal assignment

Experimental Parameter	Dietary Level			
	Control	20 ppm	80 ppm	200 ppm
Maternal Animals				
No. of Maternal Animals Assigned	**30**	**30**	**30**	**30**
Detailed Observational Battery (GD 13, 20/LD 11, 21)	**30/10**	**30/10**	**30/10**	**30/10**
Offspring				
Set C; Detailed Observational Battery [PND 4, 11, 21, 35(±1), 45(±1), 60(±2)]	**16 (min. 10)/sex**	**16 (min. 10)/sex**	**16 (min. 10)/sex**	**16 (min. 10)/sex**
Set A; Motor Activity [PND 13, 17, 21, 60(±2), 120(±5)]	**15-16 (min. 10)/sex**	**14-16 (min. 10)/sex**	**15-16 (min. 10)/sex**	**15-16 (min. 10)/sex**
Set B; Auditory Startle Habituation [PND 23, 60(±2)]	**16 (min. 10)/sex**	**16 (min. 10)/sex**	**16 (min. 10)/sex**	**16 (min. 10)/sex**
Set C; Learning and Memory [PND 22/29, 60 /67(±2)]	**15-16 (min. 10)/sex**	**16 (min. 10)/sex**	**15-16 (min. 10)/sex**	**16 (min. 10)/sex**

Experimental Parameter	Dietary Level			
	Control	20 ppm	80 ppm	200 ppm
Set D; Brain Weight **PND 21** **PND 75(±5)**	 **10/sex** **10/sex**	 **10/sex** **10/sex**	 **10/sex** **10/sex**	 **10/sex** **10/sex**
Set D; Neuropathology **PND 21** **PND 75(±5)**	 **10/sex** **10/sex**	 **10/sex** **10/sex**	 **10/sex** **10/sex**	 **10/sex** **10/sex**
Whole brain assay for Deltamethrin	Max. 6 litters	Max. 6 litters	Max. 6 litters	Max. 6 litters

The method of animal assignment minimized potential problems related to litter effects, by using at least one pup/litter. For FOB and motor activity testing, the same individual animals were evaluated at all scheduled time points. For the selection of animals and testing paradigms for cognitive (learning and memory) assessment, the same animals were used for assessments at the weanling and adult ages, but different tests were used at the two ages.

The rationale for dose selection was based, in part, on the results of a two-generation reproduction toxicology study in Sprague-Dawley rats, at dietary levels of 0, 5, 20, 80 and 320 ppm ([redacted]; 1992; M-149348-01-1). In the P-generation females, the 320 ppm dietary level was associated with severe toxicity, including decreased body weight gain beginning on day 8 of exposure, one death (prior to mating) and clinical signs (during lactation). Effects in the F1-generation before weaning included reduced body weight at birth and throughout lactation, as well as pup deaths before weaning. These results indicate that 320 ppm is clearly too toxic for use in a developmental neurotoxicity (DNT) study. No compound-related findings were evident at lower dietary levels. The pilot study ([redacted], [redacted]; 2006; M-276949-01-1) performed at 250 ppm previously summarized demonstrated an increased incidence of pup loss, including cannibalization by the dam, during the first week of lactation, indicating excessive toxicity to either the dam or offspring. This outcome supports dietary administration in the DNT study.

Based on these combined results, the dietary levels selected for the present study were 0, 20, 80 and 200 ppm, with adjustments during lactation to maintain a consistent dosage throughout the period of exposure. The 200 ppm dietary level was selected as a maximum dose the animals will tolerate without excessive toxicity. The 80 ppm dietary level was selected as an intermediate dose that may produce effects that can be compared to the reproduction study and to assist in establishing compound-related effects. Finally, the 20 ppm dietary level was selected to establish an overall NOAEL in the offspring, with minimal or no effects on the dam.

3. Test substance preparation and analysis

Concentrations of the test substance in the diet were measured by LC-MS/MS, using four batches of feed that were used in this study. The stability (at room temperature and freezer conditions) and homogeneity of the test substance in the feed were verified at dietary levels of 3 and 1000 ppm, which bracket those used in this study. Four dose groups were administered the test substance in the diet at nominal concentrations of 0, 20, 80 or 200 ppm. Formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet [redacted] Rodent [redacted] in meal form and were stored at freezer (-20 °C) conditions. Dietary concentrations were not adjusted to correct for purity (percent active ingredient) in the test substance but were adjusted (reduced) during lactation, relative to gestation, to maintain a more constant level of exposure (mg/kg/day). After day 21 of postnatal development, untreated feed was provided for consumption to all groups. A given

batch of feed was available for *ad libitum* consumption for a period of one (GD0 - LD21) or two (post-weaning) weeks prior to changing, at which time any uneaten feed was collected and disposed of by incineration.

Table 5.7.1-05: Adjustment of Dietary Concentration of Deltamethrin During Lactation

Feeding Period	Dietary Concentrations (ppm)			
Gestation	**0**	**20**	**80**	**200**
Lactation W1	**0**	**11**	**42**	**105**
Lactation W2	**0**	**9**	**35**	**87**
Lactation W3	**0**	**7**	**29**	**71**

Homogeneity and Stability Analysis:
The homogeneity and stability of the test substance in rodent feed was verified at dietary concentrations of 3 and 1000 ppm and were determined to be homogeneous and stable for seven days at room temperature (average temperature 22 ± 4 °C) and 42 days at freezer conditions (average temperature -22 ± 4 °C).

Concentration Analysis:
For **gestation**, the nominal 20, 80 and 200 ppm dietary levels averaged 106%, 105% and 107% of the nominal concentrations, respectively. Based on these results, the average dietary levels during gestation for this study were 0, 21.2, 84.0 and 213 ppm, respectively. For **lactation**, dietary levels were adjusted to achieve a more consistent dosage (mg/kg/day) throughout the period of exposure, since food consumption increases during this time period. The nominal dietary levels are indicated in the table above. During the first week of lactation dietary levels averaged 103%, 109% and 102% of the nominal concentrations, respectively and 94%, 96% and 93%, respectively, during the third week of lactation.

C. Methods

1. In-life observations

a. Maternal animals:

1) Clinical Observations

Following acclimation and continuing until animals were removed from the study (females only) or completion of co-housing (males only), P generation males and females were observed (cage-side) for clinical signs at least once daily. These observations were sufficient to characterize mortality, moribundity, behavioral changes, and overt toxicity by viewing the animal in its cage. At the discretion of the observer, animals were removed from the cage for a more detailed examination.

2) Detailed Observations

A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through LD 21. These observations were performed by an individual who was aware of the animal's dosage group assignment.

3) Detailed Observational Battery

Animals that were presumed to be pregnant (approximately 30 per dietary level) were observed on GD 13 and GD 20 and a minimum of 10 dams/dietary level that were maintained on study with suitable litters were also observed on LD 11 and LD 21. All observations were performed by individuals who were unaware of each animal's dose group assignment. This evaluation was performed under standard animal room conditions (temperature, relative humidity, etc.) and included observations in the home cage, during handling, and outside the home cage in an open field, using standardized procedures. This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviors, and posture and gait abnormalities.

4) Body Weight and Food Consumption

Body weight and food consumption were measured once weekly during gestation and lactation, as follows: GD 6–13, GD 13–20, LD 0–7, LD 7-14, and LD 14–21. In addition, dams were weighed on LD 4. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation. Fresh feed and clean feeders were provided weekly.

5) Delivery and Culling

Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated LD 0 for the dam and PND 0 for the pups. Litter size (the number of pups delivered) and pup "status" at birth were recorded for each litter. Dams that delivered fewer than three pups per sex or litter size of fewer than seven pups were sacrificed (along with their litter) without necropsy examination. For litters that met the minimum size requirements, the size of each litter was adjusted on PND 4 to yield, as closely as possible, four males and four females. When the number of male or female pups was less than four, a partial adjustment was made (e.g., three females and five males). When there were more than 23 acceptable litters for any dietary level, the surplus litters were sacrificed on PND 4 after weighing without routine necropsy, with preference given to retaining litters with a full complement of four males and four females. Culled dams and pups were sacrificed by carbon dioxide (CO_2) asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by CO_2 asphyxiation.

6) Moribund Animals and Animals Found Dead

There were no P-generation males or females found moribund or dead while on study.

7) Termination

Males: Following co-habitation, males were sacrificed by CO_2 asphyxiation and discarded unless an alternative use was found.
Females: Dams were sacrificed on LD 21, by CO_2 asphyxiation, following the weaning of their respective litters. Females that were sperm positive and/or had an internal vaginal plug, but did not deliver, were generally sacrificed on GD 24 without necropsy examination.

b. Offspring

1) Litter Observations

The day of completion of parturition was designated as LD (PND) 0. As soon as possible following parturition, pups were examined for ano-genital distance to establish their gender, and then tattooed and weighed. Live pups were counted, sexed, and weighed individually for each litter on PND 0, 4, 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or moribundity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily (a.m.) before weaning and once weekly thereafter. These observations were performed by an individual who was aware of assignments to dose level.

2) Developmental Landmarks

Beginning on postnatal day 38, male offspring were examined daily for balanopreputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset was recorded. On PND 21, all pups were also tested for the presence of pupil constriction.

3) Postweaning Observations

After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly, as well as on the day that vaginal patency or balanopreputial separation was achieved.

4) Body Weight and Food Consumption

Surviving pups were weighed on PND 0, 4, 11, 17, and 21, and once weekly thereafter. The individual pups were also weighed when vaginal patency or balanopreputial separation was first evident. Food consumption was not measured after weaning on PND 21, when all animals received untreated diet.

5) Neurobehavioral Evaluations

Observations and the schedule for those observations are summarized as follows. The test room used for motor activity, auditory startle habituation, and passive avoidance conditioning was a standard animal room set to be maintained on the same light/dark cycle as the room in which animals were housed, with tests conducted during the light phase. The water maze testing was performed in the room where animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device. One exception was that animals were purposely tested in the same water maze on both occasions, as per standard procedure. Males and females were tested on the same days at the appropriate days of age. After sexual maturation, test devices were cleaned during the ensuing interval to reduce the residual scent from the other gender.

- Functional Observational Battery (Set C): On postnatal days 4, 11, 21, 35 (±1 day), 45(±1 day), and 60 (±2 days), approximately 16 offspring/sex/group (minimum one male or one female from each litter) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage involved. This evaluation was performed according to the procedures described for maternal animals (see above), using standardized procedures. The only difference is that the neonates (i.e., PND 4 and 11) were not evaluated in the open field, since this is routinely done only if the observer considers it necessary for evaluation and this was not the case in the present study.
- Motor Activity Testing (Set A): Motor activity was measured for approximately 16 rats/sex/dose (minimum 10/sex/dietary level) on PND 13, 17, 21, 60 (±2 days) and 120 (±5 days). Animals were tested at 120-days of age to address possible questions raised by published findings in mice that were tested at this age, following exposure to deltamethrin

during lactation. The same offspring were evaluated in the figure-eight maze for 60 minutes at each time point, using a computer-automated system (Columbus Instruments, Columbus, OH) and personal computer for automated data collection. The figure-eight maze was selected as an established and widely-used automated activity device that can be used to detect both increases and decreases in activity. Each maze consisted of a series of inter-connected alleys (approximately 10 x 10 cm in cross-section) converging on a central arena and covered by transparent acrylic plastic. Each maze had eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys) to measure activity and an activity count was registered each time a beam was interrupted. The floor of each maze rested above absorbent paper which was changed routinely at the end of each day. A Columbus Instruments (Columbus, OH) Universal Maze Monitoring System and a personal computer were used for automated data collection. Broad-spectrum background noise [74+2dB(A)] was provided throughout the test to minimize acoustical variations during testing. The uniformity of light intensity (100+70 Lux) over each maze was verified daily. Motor and locomotor activity were examined as total activity counts (beam interruptions) for the 60-minute session and as activity during each ten-minute interval. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Thus, for locomotor activity, only one interruption of a given beam was counted until the rat relocated in the maze and interrupted a different beam. Habituation was evaluated as a decrement in activity over consecutive intervals of the test session.

- Auditory Startle Reflex Habituation (Set B): Auditory startle reflex habituation testing was performed in approximately 16 rats/sex/dose (minimum 10 offspring/sex/dose)) on PND 22 and 60 (±2 days), using an automated system. A personal computer was used to control the operation of an integrated startle response test system ([redacted]) and for automated data collection. Groups of four animals (maximum) were tested simultaneously within each of two startle system enclosures. Each enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material, and housed a speaker mounted in a central position within the ceiling of the enclosure to provide the eliciting stimulus (S2) - a 50-msec burst (0 msec rise/fall) of broad-spectrum "white" noise [approximately 118 dB(lin)]. Each enclosure also housed four load cell/force transducer assemblies that are designed to measure the startle response. During the test session, animals were placed into individual restraining cages that are positioned on top of each load cell. The test session consisted of 50 trials that began following approximately a 5 min adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-second intervals. The peak response amplitude was determined for each trial as described below. The average response amplitude and the magnitude of decrease (habituation) over blocks of ten trials were compared among the dosage groups. Data collection began with the presentation of S2 and continued thereafter for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (i.e., one sample/msec for 200 msec) and converted to grams using a previously determined calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's individual response curve. Baseline was defined as the average force (g) exerted on the platform during the first 8 msec following the onset of S2, a time period that precedes response onset. This baseline value was taken to represent an approximate body weight measurement that was used to verify that the equipment

used to measure the response amplitude was functioning properly. Response amplitude was defined as the maximum value of the average curve, minus the baseline (i.e., removing the animal's body weight from the measurement). Latency to peak was the time (msec) following the onset of S2 when the peak response amplitude occurs.

- Learning and Memory Testing (Set C): Learning and memory testing were performed in approximately 16 rats/sex/dietary level, minimum 10 offspring/sex/dose. The same set of animals were used for testing passive avoidance (on PND 22 and 29) and water maze [PND 60 (± 2 days) and again seven days later].

Postweaning - Passive Avoidance

Animals were tested for acquisition on PND 22 and for retention on PND 29. Testing was conducted using equipment and computer programs from [redacted]. A personal computer was used to control the operation of the equipment and for automated data collection. Testing took place in individual isolation cubicles, each housing a single shuttle cage. Each isolation cubicle was lined with foam insulation to attenuate sound in the chamber and had a fan with a baffled air intake and exhaust system for ventilation. The shuttle cage consisted of a Plexiglas and stainless-steel rectangular chamber fitted with front-loading access. Each shuttle cage (14 inches wide x 7 inches deep) was separated into two compartments of equal size (approximately 7 x 7 inches) by a wall that supported a centrally-located sliding (guillotine-type) door. The two compartments were identical, except that the walls in one compartment were lined with black film (dark-side) and the walls in the other compartment were not lined and it was illuminated during the test with a high-intensity lamp. The lamp was switched on to illuminate the light compartment at the start of each trial and remained on until either the animal crossed to the dark compartment or the trial ended. The floor of the cage consisted of a grid of stainless-steel bars. The movement of the animal from the starting (light) side to the dark compartment was detected by a photocell system. [redacted] solid-state scanning shock generator was used to deliver a brief (0.5 sec) pulse of mild (0.5 mA) distributed shock to the grid floor when the animal crossed to the dark compartment.

The test was conducted according to established procedures. After adaptation, individual animals were placed individually into the "lighted" compartment of a conditioning apparatus (the shuttle cage), facing toward the light. After approximately 60 seconds, the trial began with the light being illuminated to signal the beginning of the trial and the door separating the two compartments opening, so that each rat was provided access to the non-illuminated side of the cage. When the rat crossed into the dark compartment, the door automatically closed, the shock was delivered, and the light switched off - signaling the end of that trial. At that time, the animal was returned promptly to the holding cage to wait for the next trial. If the rat failed to cross within 180 sec, it was returned to the holding cage and the latency assigned an arbitrary score of 180. This restriction dictated the use of nonparametric statistical analyses. The procedure was repeated until either the rat remained in the lighted compartment for 180 sec on two consecutive trials or until 15 trials had elapsed, whichever occurred first. Rats that failed to meet the criterion during the learning phase were assigned a value of 15 for the trials-to-criterion variable.

The test was repeated one week later. For this second trial, rats were placed in the illuminated side of the apparatus, given a 20-sec acclimation period, and the latency to enter the dark side recorded. Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2

(learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring - Water Maze

The animals assigned to passive avoidance testing were also assigned to water maze testing. Animals were tested on postnatal day 60 (+2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 ±1°C. The mazes were constructed of opaque Plexiglas, with corridors approximately five inches wide and walls approximately 16 inches high with approximately 7.5 inches of water. This maze was selected as an established and widely-used device that can be used to measure associative learning and memory.

On each test trial, the rat was placed into the starting position at the base of the M-maze stem, located between the two lateral arms. On the first (learning) trial, the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the water and then removed from the maze. The initial arm chosen on this learning trial was designated the incorrect goal during the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any given trial were guided to the correct goal with the exit ramp and then removed from the water. Between trials, the animal was returned to a transport cage to wait for the next trial. The inter-trial interval was approximately 15 (+5) seconds. Each rat was required to reach a criterion of five consecutive error-less trials to terminate the test session. The maximum number of trials in any test session was fifteen. Latency (in seconds) to choose the correct goal or the maximum 60-second interval was recorded for each trial, as was the number of errors (incorrect turns in the maze) during each trial.

Animals that satisfied the above criteria within the 15-trial limit were tested for retention seven days following acquisition (animals that failed to reach criterion during acquisition were excluded from the retention phase of the experiment). The correct goal and the criterion were the same for both sessions.

Dosage groups were compared for the following dependent measures: Measures for acquisition included the number of trials-to-criterion, the average number of errors (incorrect turns in the maze) for each trial, and the latency (in seconds) to reach the correct goal on trial 2 (a measure of short-term retention). Measures for retention included the number of trials-to-criterion, the average number of errors for each trial, and the latency (in seconds) to reach the correct goal on trial 1 (a measure of long-term retention).

6) Ophthalmology

At approximately 50–60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level, representing at least 20 litters per level) that were selected for perfusion at study termination. The exam took place in a semi-darkened room. The pupillary reflex was tested using a penlight or transilluminator, with a mydriatic agent applied to each eye to dilate the pupil. The conjunctiva and cornea were examined with a slit lamp microscope either before or after pupillary dilatation. After mydriasis, vitreous humor, retina, choroid, and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

2. Postmortem observations:

Maternal Animals: Maternal animals were sacrificed by CO_2 asphyxiation on Day 21 of lactation following the weaning of their respective litters. The dams were discarded without postmortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed by CO_2 asphyxiation on or after GD 24 without necropsy examination.

Offspring:

Necropsy: The offspring selected for brain weight or neuropathological evaluation (Set D) were sacrificed on PND 21 or 75 (± 5 days). In addition, randomly selected animals from Sets A–C that were used to measure fresh brain weight were sacrificed by CO_2 asphyxiation and underwent a necropsy examination. The necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded.

Perfusion: Animals selected for perfusion on PND 21 (from Set D) or at study termination (from Sets A–C) were deeply anesthetized using an intraperitoneal dose of pentobarbital (approximately 50 mg/kg) and then perfused via the left ventricle with a sodium nitrite (in phosphate buffer) flush followed by in situ fixation using universal fixative (1.0% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde)] in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial, and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs, and physical identifier were collected. All tissues were post-fixed in 10% buffered formalin, with the exception of the eyes, which were post-fixed in Davidson's fixative. The brain was weighed upon removal from the skull, prior to placement into formalin, and the brain:body weight ratio calculated.

Measurements: At necropsy and prior to placement in 10% formalin, a Vernier caliper was used to obtain two linear measurements (mm).

1. Anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; and
2. Anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole.

These gross measurements were performed by an individual who was aware of dose group assignments.

Histology: The brain tissue from perfused animals and any gross lesions collected at necropsy were further processed for microscopic examination. After the gross measurements were taken, the brain was divided into eight coronal sections for microscopic examination. The eight brain sections were processed according to standard procedures for paraffin embedding, sectioned at approximately 5 µm, and examined after staining with hematoxylin and eosin (H&E). In addition, the brain sections (duplicate sections) reserved for morphometric measurements (levels 3-5, and 7) were stained using luxol fast blue/cresyl violet. Additional tissues were collected for microscopic examination from animals that were perfused at study termination. This included three levels of the spinal cord (cervical, thoracic, and lumbar), the cauda equina, eyes, optic nerves, and gastrocnemius muscle embedded in paraffin and stained with H&E. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol methacrylate (GMA). GMA-embedded tissues were sectioned at 2–3 µm and stained using a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial, and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

The checked (x) tissues were evaluated for adult offspring.

	CENTRAL NERVOUS SYSTEM		**PERIPHERAL NERVOUS SYSTEM**
	BRAIN (8 levels, including)		PERIPHERAL NERVES
x	Forebrain	x	Sciatic
x	Center of cerebrum	x	Tibial
x	Midbrain	x	Sural
x	Cerebellum		
x	Pons		
x	Medulla oblongata		
	SPINAL CORD		OTHER
x	Cervical swelling	x	Lumbar dorsal root ganglion
x	Thoracic	x	Lumbar dorsal root fibers
x	Lumbar swelling	x	Lumbar ventral root fibers[1]
		x	Cervical dorsal root ganglion
	OTHER	x	Cervical dorsal root fibers[1]
x	Gasserian Ganglion	x	Cervical ventral root fibers[1]
x	Optic nerves	x	Gastrocnemius muscle
x	Eyes		
x	Cauda equina		

[1] Dorsal and ventral root fibers were evaluated as they were generally included with the ganglion

Micropathology and Morphometry: The tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion was evident, further analysis was not performed. Any region where treatment-related neuropathology was observed underwent the following semi-quantitative analysis. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency of each type of lesion was determined with the severity of each lesion graded. The code was then broken and the data evaluated for dose-effect relationships.

Selected brain regions underwent the following quantitative analysis, with the individual performing the measurements aware of dose assignments. Initially, eight linear measurements were taken. Two of the seven measurements involve gross measurements of the intact brain, as described above. The other five were taken from the histologic sections using software calibrated with an ocular micrometer. These five measurements are described as follows:

1. Frontal cortex thickness (Forebrain). This measurement was of the dorsal portion of the cerebral cortex within the coronal section passing through the region of the optic chiasm.
2. **Parietal cortex** thickness (Forebrain). This measurement was of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.
3. **Caudate putamen** horizontal width (Forebrain; maximum cross-sectional width). This measurement was performed on the coronal section taken at the level of the optic chiasm.
4. Hippocampal gyrus thickness (Midbrain). This measurement was of the full width of the hippocampal gyrus, from the ventral tail of the dentate gyrus to the overlying subcortical

white matter. Measurements were taken from the hippocampus from both sides of this section, and the mean value recorded.

5. Cerebellum height (Cerebellum/Pons). This measurement extends from the roof of the fourth ventricle to the dorsal surface.

In addition to these measurements, all brain sections from these control and high-dose male and female offspring underwent an extensive micropathologic evaluation

D. Data Analysis

1. Statistical Analysis

Statistical evaluations were generally performed using software from INSTEM Computer Systems, TASC, or SAS. The level of significance was set at $p \leq 0.05$, with the exception of Bartlett's Test, which was tested at $p \leq 0.001$. In general, continuous data was initially assessed for equality of variance using Bartlett's Test. Group means with equal variances were analyzed further using an Analysis of Variance (ANOVA), followed by a Dunnett's Test if a significant F-value was determined in the ANOVA. In the event of unequal variances, these data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U Test for between-group comparisons).

Detailed observational b

Reproductive Indices:

The following reproductive indices were calculated from breeding and parturition records of animals in the study:

Mating Index = (no. of insemi **attery,** continuous data were analyzed using an ANOVA, with post-hoc comparisons using Dunnett's Test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling (CATMOD) Procedures, with post-hoc comparisons using Dunnett's Test and an Analysis of Contrasts, respectively.

Motor and locomotor activity (total session activity and activity for each 10 min interval) was analyzed using ANOVA procedures. Session activity data for the four test occasions were analyzed using an ANOVA to determine whether there was a significant day by treatment interaction. For days on which there was a significant treatment effect, Dunnett's test was used to determine whether the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine whether there was a significant treatment by interval interaction on each test occasion. For those test days, the data for each interval was subjected to analysis using Dunnett's test to determine whether the treated group was significantly different from the control.

Auditory startle response amplitude data (peak amplitude) for the three test occasions were first analyzed using an ANOVA procedure. If there was a significant group effect, Dunnett's test was used to determine whether the treated group was significantly different from control. The response amplitude data for each block of ten trials (five blocks/test session) were subjected to a Repeated-Measures ANOVA using test block as the repeated measure. If there was a significant group by block interaction, the values for each block were subjected to analysis using Dunnett's test to determine if the results for treated animals were significantly different from control.

Passive avoidance data were analyzed as follows. Latency data were analyzed using a Wilcoxon Test for time to failure (i.e., time to cross). The number of trials-tocriterion was analyzed using Kruskal-

Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning (acquisition) phase was analyzed as incidence data.

Water maze results were analyzed using parametric and non-parametric tests. Latency data were analyzed by a univariate ANOVA, with post-hoc analysis using Dunnett's test. The number of trials-tocriterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats failing to meet the criterion level of performance in the learning phase was analyzed as incidence data.

Pathology data were screened for potential effects and then evaluated using the following approach. Additional statistical tests to assess continuous and frequency data may have been used when deemed appropriate.

Table 5.7.1-06: Statistical Analyses

DATA TYPE	DATA	STATISTICAL TESTS	COMPUTER
	Organ Weight	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)	DATATOX
	Gross Brain Measurements	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)	DATATOX
	Microscopic Brain Measurements	ANOVA and/or *t*-Test (2)	Corel Quattro Pro
FREQUENCY	Ophthalmology	Visually Screened (3)	Trend.exe
	Gross Pathology	Visually Screened (3)	DATATOX/Trend.exe
	Micropathology	Chi-Square Fisher's Exact Test	SAS

All statistical tests based significance level of $p \leq 0.05$, except for Bartlett's, which is based on $p \leq 0.001$.
(1) ANOVA used if data were homogeneous; Kruskal-Wallis used if data were non-homogeneous
(2) A *t*-Test, 2-tailed, used for two-group comparisons; an ANOVA was used for multiple-group comparisons
(3) If potential compound effects were suspected, then Chi-Square and one-tailed Fisher's Exact Tests were used.

2. Indices
nated females/no. of females co-housed with males) X 100
Fertility Index = (no. of pregnant females/no. of inseminated females) X 100

Offspring Viability Indices:
The following viability (survival) indices were calculated from lactation records of litters in the study:

Live Birth Index = (no. of live pups born per litter/total no. of pups per litter) X 100
Viability Index = (no. of live pups on Day 4 pre-culling per litter/no. of live pups born per litter) X 100
Lactation Index = (no. of live pups on Day 21 per litter/no. of live pups on Day 4 post-culling per litter) X 100

II. Results and discussion

A. Parental Animals

1. Mortality and clinical and functional observations

No P-generation females were found dead during gestation or lactation. There were also no P-generation males found moribund or dead after initiation of the study (males did not receive the test substance).

Compound-related clinical signs were not evident at any dietary level during gestation. Findings that are considered incidental and unrelated to treatment included scab formation in three high-dose females and areas of hair loss (alopecia) in two low- and one high-dose females.

Compound-related clinical signs were not evident at any dietary level during lactation. Findings that are considered incidental and unrelated to treatment included scab formation in two high-dose females and areas of hair loss (alopecia) in one or two females, each from various dose groups, with no relationship to dietary level.

Table 5.7.1-07: Mortality and Clinical Signs in Maternal Animals

Observation	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Gestation (Days 6–21)				
No. of Females Examined on GD 6	30	30	30	30
Scab formation	0	0	0	3
Hair Loss	0	2	0	1
No. of Females Found Dead	0	0	0	0
Lactation (Days 0–21)				
No. of Females Examined on LD 0 [a]	28	29	30	30
Scab formation	0	0	0	2
Hair loss	0	2	1	2
No. of Females Found Dead	0	0	0	0

There were no test substance-related findings observed in the detailed observational battery in dams at any dietary level. Findings that are considered incidental and unrelated to treatment included (areas of) alopecia in various dose groups, with no relationship to dietary level, and a dermal lesion described as a scab in one high-dose animal.

Table 5.7.1-08: Maternal Functional Observations

Observation	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Gestation Day 13				
No. of Animals Examined	30	30	30	30
Handling-Other; Alopecia				
Not Observed	30(100)	29(97)	30(100)	29(97)
Present	0(0)	1(3)	0(0)	1(3)
Gestation Day 20				
No. of Animals Examined	30	30	30	30
Handling-Other; Alopecia				
Not Observed	30(100)	28(93)	30(100)	29(97)
Present	0(0)	2(7)	0(0)	1(3)
Handling-Other; Scab				
Not Observed	30(100)	30(100)	30(100)	29(97)
Present	0(0)	0(0)	0(0)	1(3)
Lactation Day 11				
No. of Animals Examined	10	10	10	10
Handling-Other; Alopecia				
Not Observed	10(100)	10(100)	10(100)	9(90)
Present	0(0)	0(0)	0(0)	1(10)
Lactation Day 21				
No. of Animals Examined	10	10	10	10
Handling-Other; Alopecia				*
Not Observed	10(100)	10(100)	9(90)	9 (90)
Present	0(0)	0(0)	1(10)	1 (10)

2. Body Weight and Food Consumption

Gestation: Body weight and weight gain were significantly reduced by treatment in high-dose animals. These differences in body weight were statistically significant on GD 13 and GD 20 (6-7%), with weight gain reduced an average 17% from GD 0-20 for high-dose females, compared to controls. In addition, food consumption was significantly reduced (17%) in highdose animals, compared to controls, on GD 6-13. Body weight, weight gain and food consumption were not affected by treatment at lower dietary levels.

Lactation: On LD 0-7, body weight (6-8%) and food consumption (9%) were significantly reduced in highdose animals, compared to controls, but were comparable to control at lower dietary levels. Subsequent measures of body weight and food consumption were comparable to control at all dietary levels.

Table 5.7.1 09: Mean (±S.E.) Maternal Body Weight and Food Consumption

Observations/Study Week	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Gestation				
Mean Body Weight (g) GD 0	213.5±1.78 (28)	210.9±1.74 (29)	208.1±2.76 (30)	209.2±1.93 (30)
Mean Body Weight (g) GD 6	237.3±2.24 (28)	234.3±2.02 (29)	231.3±2.98 (30)	232.5±2.26 (30)
Mean Body Weight (g) GD 13	263.7±2.62 (28)	260.1±2.38 (29)	255.3±3.42 (30)	247.1**±2.64 (30)
Mean Body Weight (g) GD 20	326.1±3.68 (28)	320.2±3.19 (29)	306.8±5.02 (30)	302.7**±3.24 (30)
Mean Weight Gain (g) GD 0–20	112.6±2.24 (28)	109.3±2.22 (29)	108.7±2.93 (30)	93.6**±2.15 (30)
Mean Food Consumption (g/animal/day) GD 6–13	20.7±0.47 (28)	19.9±0.31 (28)	19.6±0.43 (30)	17.2**±0.47 (30)
Mean Food Consumption (g/animal/day) GD 13–20	22.0±0.42 (27)	21.9±0.36 (28)	23.0±0.80 (30)	21.6±0.50 (30)
Lactation				
Mean body weight (g) LD 0	252.3±3.46 (28)	248.9±2.45 (29)	241.1±4.07 (30)	233.5**±2.47 (30)
Mean body weight (g) LD 4	270.3±2.85 (28)	264.0±3.19 (26)	258.3±4.22 (28)	248.5**±3.05 (26)
Mean body weight (g) LD 7	276.3±3.01 (23)	275.2±3.71 (23)	269.0±5.12 (23)	258.8**±3.12 (23)
Mean body weight (g) LD 14	288.6 ±3.10 (23)	288.5±2.80 (23)	286.8±5.07 (23)	280.1±3.16 (23)
Mean body weight (g) LD 21	284.8±2.97 (23)	285.9±3.19 (23)	285.1±4.21 (23)	278.6±3.27 (23)
Mean food consumption (g/animal/day) LD 0–7	36.3±1.30 (23)	34.7±1.31 (22)	35.1±2.00 (23)	33.2±1.40 (23)
Mean food consumption (g/animal/day) LD 7–14	51.1±1.05 (22)	48.7±0.94 (22)	48.8±1.26 (22)	49.2±1.44 (22)
Mean food consumption (g/animal/day) LD 14–21	65.4±2.10 (23)	62.0±3.35 (23)	62.8±1.37 (22)	61.3±1.24 (22)

Values are mean ± S.E. (*n*). Means for gestation include only dams found sperm positive and with pups at termination of gestation.
** Statistically different from control, Dunnett's Test $p \leq 0.01$

3. Test substance intake

The average daily intake of the active ingredient (A.I.) (mg A.I./kg body weight/day) was calculated using weekly body weight and food consumption data. The general relationship used for this calculation was: [AI in feed (ppm)/1,000] x [feed consumed (g/kg body wt/day)] = mg AI/kg body wt/day. The average consumption of test substance for females that received diets containing nominal concentrations of 0, 20, 80, or 200 ppm during gestation, with adjustments during lactation, are presented in Table 5.7.1-10. Based on these results, the average daily intake of active ingredient during gestation and lactation was 0, 1.64, 6.78, and 16.1 mg/kg/day.

Table 5.7.1-10: Mean Maternal Test Substance Intake (mg/kg body weight/day)[1]

Period	Dietary Level of Deltamethrin		
	20 ppm	80 ppm	200 ppm
Gestation			
Gestation Days 6–13	1.8±0.02 (28)	7.1±0.12 (30)	15.7±0.37 (30)
Gestation Days 13–20	1.8±0.02 (28)	7.5±0.22 (30)	18.6±0.40 (30)
Lactation			
Lactation Days 0–7	1.6±0.07 (22)	6.6±0.41 (23)	15.2±0.57 (23)
Lactation Days 7–14	1.6±0.04[2] (23)	6.6±0.13[2] (22)	16.6±0.47[2] (22)
Lactation Days 14–21	1.4±0.08 (23)	6.1±0.12 (22)	14.4±0.25 (22)

1 Values are mean ± standard error (n). Dietary concentrations were reduced during weeks 1-3 of lactation (by factors of 1.9, 2.3 and 2.8, respectively), based on estimated increases in feed consumption (g consumed/kg body wt. day) during lactation).
2 Value estimated, based on the average percent nominal concentration measured for all other weeks.

4. Reproductive performance

There were no effects on reproductive parameters at any dietary level (Table 5.7.1-11).

Table 5.7.1-11: Reproductive Performance

Observation	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
No. of Animals Co-housed[a]	30	30	30	30
No. of Animals Mated	30	30	30	30
Maternal Wastage				
No. of Dams not Pregnant	2	1	0	0
No. of Dams that Delivered Dead Pups	0	1	1	0
No. of Dams with Pre-Mature Delivery	0	0	0	0
Mating Index	100.0	100.0	100.0	100.0
Fertility Index (No. of pregnant females/No. of inseminated females X 100)	93.3	96.7	100.0	100.0
Gestation Length (days)[b]	21.8±0.10 [22.0] (21.0–23.0)	21.8±0.11 [22.0] (21.0–23.0)	21.7±0.12 [22.0] (21.0–23.0)	21.7±0.12 [22.0] (21.0–23.0)

[a] **Number of animals assigned to each dietary level.**
[b] **Values are mean ± S.E., [median], and (range).**

5. Maternal postmortem results – not applicable to the present study.

B. Offspring

1. Viability and clinical signs

Litter parameters and pup viability were not affected by the test substance at any dietary level (Table 5.7.1-12).

Table 5.7.1-12: Litter Size and Viability

Observation	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
No. of Litters	23	23	23	23
Total No. of Pups Born	265	249	259	258
Total No. of Pups Missing	1	6	2	0
Litters with Pups Missing	1	2	2	0
Total No. of Pups Found Dead	0	1	1	1
Litters with Pups Found Dead	0	1	1	1
Total No. of Pups Cannibalized	0	0	0	0
Litter with Pups Cannibalized	0	0	0	0
Litter Size	11.5±0.27 [11.0] (9.0–14.0)	10.8±0.38 [11.0] (7.0–14.0)	11.3±0.30 [11.0] (8.0–14.0)	11.2±0.41 [11.0] (7.0–16.0)
Stillborn Pups				
Number	0	3	0	0
%	0.0	1.2	0.0	0.0
Mean±S.E.	0.0±0.00	0.1±0.13	0.0±0.00	0.0±0.00
[Median]	[0.0]	[0.0]	[0.0]	[0.0]
(Range)	(0.0–0.0)	(0.0–3.0)	(0.0–0.0)	(0.0–0.0)
Mean No. of Viable Pups				
Birth	12	11	11	11
Day 4 (Pre-cull) [a]	12	10	11	11
Day 4 (Post-cull) [b]	8	8	8	8
Day 21	8	8	8	8
Live Birth Index [c]	100.0±0.00 [100.0] (100-100)	98.9±1.09 [100.0] (75-100)	100.0±0.00 [100.0] (100-100)	100.0±0.00 [100.0] (100-100)
Viability Index [c]	100.0±0.00 [100.0] (100-100)	97.9±1.83 [100.0] (58–100)	98.8±0.64 [100.0] (90–100)	99.7±0.29 [100.0] (93–100)
Lactation Index [c]	99.5±0.54 [100.0] (88–100)	99.5±0.54 [100.0] (88–100)	100.0±0.00 [100.0] (100–100)	100.0±0.00 [100.0] (100–100)

[a] Before standardization (culling) [b] After standardization (culling) [c] Values are mean ± S.E., [median], (range)

Postpartum (PND 0-21). There were no compound-related signs during lactation in males or females at any dietary level. Incidental findings that were evident on occasion in several individuals from various dose groups, including control, were limited to bruising on the face, back and/or body.

Post Weaning. There were no compound-related clinical signs after weaning (when exposure was discontinued) in males or females at any dietary level. Findings considered incidental and unrelated to treatment included urine stain, perianal stain, red nasal stain, general ocular opacity, exophthalmus and dehydration. Also, areas of alopecia, rough coat and minor dermal lesions were evident in individual control and treated males and/or females. These findings occurred after treatment had been discontinued, in some cases occurred in control animals and did not occur in a dose-related pattern to indicate a residual compound-related effect. Lastly, one low-dose male had a firm dermal palpable mass located on the right side of the abdomen on PND 43 and was later found dead on PND 56. This finding is considered incidental and unrelated to treatment because there was no correspondence with dietary level, occurred after treatment had been discontinued and was only observed in this one animal.

Animals Found Dead or Moribund (Post-Culling). The number of offspring (males and females combined) found dead, moribund, or missing after culling litters on PND 4 was 2, 3, 0 and 0 for the control, low-, mid- and high-dose levels, respectively. Thus, there was no correspondence with dietary level.

2. Body weight

Preweaning Body Weight:

There was no difference in birth weight at any dietary level. The first indication of a difference in weight was evident on PND 4, with a statistically lower body weight for high-dose females (9%; pre-culling group) and a similar trend developing in high-dose males (pre-culling group, both sexes combined, was significantly reduced by 9% and post-culling group of males was significantly reduced by 9%). Beginning on PND 11 and continuing through PND 21, body weight for high-dose males and females was statistically reduced (7-10% for males; 6-9% for females; 6-9% for combined males and females), compared to controls. Lastly, body weight gain from PND 0 -11 for high-dose males and females was significantly reduced (10-15% for males; 10-18% for females; 10-18% for combined males and females), compared to controls.

Table 5.7.1-13: Mean (± S.E.) Preweaning Pup Body Weights (g)

Postnatal Day	Dietary Level of Deltamethrin							
	Control	20 ppm	80 ppm	200 ppm	Control	20 ppm	80 ppm	200 ppm
	Males				Females			
0	6.0±0.09 (23)	6.1±0.10 (23)	5.9±0.09 (23)	5.8±0.12 (23)	5.7±0.09 (23)	5.8±0.10 (23)	5.6±0.09 (23)	5.5±0.11 (23)
4 [a]	9.9±0.18 (23)	10.4±0.24 (23)	9.6±0.25 (23)	9.1±0.30 (23)	9.6±0.19 (23)	10.0±0.24 (23)	9.1±0.25 (23)	8.7*±0.30 (23)
4 [b]	10.0±0.19 (23)	10.4±0.24 (23)	9.6±0.25 (23)	9.1*±0.30 (23)	9.6±0.19 (23)	10.0±0.26 (23)	9.2±0.25 (23)	8.8±0.31 (23)
11	24.8±0.48 (23)	25.4±0.50 (23)	24.2±0.52 (23)	22.4**±0.59 (23)	24.2±0.49 (23)	24.6±0.55 (23)	23.3±0.59 (23)	22.0*±0.59 (23)
17	38.0±0.61 (23)	38.4±0.68 (23)	37.2±0.76 (23)	35.4*±0.72 (23)	36.7±0.51 (23)	37.1±0.70 (23)	35.6±0.77 (23)	34.6*±0.69 (23)
21	49.2±0.88 (23)	49.1±0.81 (23)	47.9±1.04 (23)	45.5*±0.94 (23)	47.9±0.72 (23)	47.4±0.90 (23)	45.7±1.07 (23)	44.6*±0.92 (23)

Values are mean ± S.E. (*n*)

[a] Before standardization (culling). * Values were statistically different from control, $p \leq 0.05$

[b] After standardization (culling). ** Values were statistically different from control, $p \leq 0.01$

Postweaning **(Table 5.7.1-14):**

The reduced body weight that developed during lactation persisted in high-dose animals for the first one week (females) or four weeks (males) after weaning. These differences from control in high-dose males and females were statistically significant (average 4-8% and 7%, respectively).

There was no difference from control thereafter in high-dose males and females, demonstrating recovery after the discontinuation of treatment, and no difference from control at lower dietary levels at any time after weaning.

Table 5.7.1-14: Mean (± S.D.) Postweaning Pup Body Weights (g)

Postnatal Day [a]	Dietary Level of Deltamethrin							
	Control	20 ppm	80 ppm	200 ppm	Control	20 ppm	80 ppm	200 ppm
	Males				Females			
28	79.6±7.9 (23)	81.3±6.1 (23)	78.0±7.0 (23)	73.4*±7.2 (23)	78.3±6.8 (23)	79.1±5.4 (23)	75.0±6.8 (23)	73.1*±5.4 (23)
35	125.5±9.5 (23)	127.3±7.9 (23)	122.2±9.1 (23)	118.6*±8.7 (23)	113.0±7.1 (23)	115.6±5.5 (23)	110.6±7.1 (23)	109.5±5.1 (23)
42	173.3±11.9 (23)	173.7±8.9 (23)	168.5±10.4 (23)	164.3*±10.1 (23)	136.2±8.2 (23)	138.4±5.6 (23)	134.1±6.7 (23)	132.7±5.9 (23)
49	216.9±14.3 (23)	217.9±10.7 (23)	211.5±12.5 (23)	207.5*±11.0 (23)	150.6±9.3* (23)	155.1±6.3 (23)	152.8±7.5 (23)	151.5±7.0 (23)
56	260.0±17.6 (23)	261.2±14.1 (23)	253.6±15.2 (23)	249.4±13.4 (23)	170.3±10.3 (23)	172.6±7.4 (23)	167.1±9.5 (23)	167.1±7.0 (23)
63	291.9±20.3 (23)	294.4±15.2 (23)	285.5±16.8 (23)	282.4±14.0 (23)	182.1±10.1 (23)	185.9±8.0 (23)	181.1±10.6 (23)	181.8±7.5 (23)
70	321.2±23.1 (23)	324.5±16.6 (23)	314.6±20.1 (23)	311.7±13.5 (23)	191.1±11.3 (23)	195.1±8.2 (23)	190.4±11.4 (23)	191.3±7.7 (23)

Values are mean ± S.D. (*n*). Values were not statistically different from control, $p \leq 0.05$

[a] Actual days of measurement occured during the week of PND 28, 35, 42, 49, 56, 63, and 70.

* Statistically different from control, p ≤ 0.05

3. Developmental Landmarks (Sexual Maturation) and Pupil Constriction:

The onset of balanopreputial separation was significantly delayed in high-dose males (1.6 days), compared to controls, but was not affected at lower dietary levels. The average age of onset was 43.5 days for controls, compared to 44.0, 44.3 and 45.1 days for 20, 80 and 200 ppm dietary groups, respectively. This delay in high-dose males was in correlation with statistically significant decrease in body weight.

The onset of vaginal patency was not affected by treatment at any dietary level. The average age of onset was 32.0 days for controls, compared to 31.9, 32.8 and 33.4 days for 20, 80 and 200 ppm dietary groups, respectively. The modest difference from control at the highest dietary level (+1.4 days) is not attributed to treatment, because there was no statistical difference and because the average age of onset for the controls in this study was below the range of historical control while the high-dose females were well within historical control (32.6 to 34.6 days).

Pupil constriction in response to a penlight was apparent in all control and treated pups on PND21. Therefore, there was no indication of a compound-related effect at any dietary level. The data are presented in Table 5.7.1-15.

Table 5.7.1-15. Mean (± S.E.) Age of Sexual Maturation (days)

Parameter	Dietary Level of Deltamethrin			
	Control	**20 ppm**	**80 ppm**	**200 ppm**
Number of Litters (M/F)	23/23	23/23	23/23	23/23
Balanopreputial Separation % Pups Reaching Criteria	43.5±0.31 (100)	44.0±0.39 (100)	44.3±0.29 (100)	45.1*±0.48 (100)
Vaginal Opening % Pups Reaching Criteria	32.0±0.32 (100)	31.9±0.32 (100)	32.8±0.36 (99)	33.4±0.50 (100)

Values are mean ± S.E.
* Statistically different from control, p_0.05

4. Behavioral assessments:

a. Detailed observational battery:

For high-dose males, only 19 litters were represented on PND 21, 35, 45 and 60, because one male (number 3102 05) was inadvertently sacrificed on PND 21. The available number of animals (15 or 16 males and 16 females per dietary level) was considered sufficient to establish compound-related effects.

Compound-related effects were not evident in females at any dietary level. One possible compound-related effect was evident in high-dose males on PND 4, consisting of a statistically significant difference from control involving a difference in reaction to handling in the home cage, where eight of 16 high-dose males, compared to one of 16 control males, minimally resisted handling with vocalizations. There were no effects related to treatment in males at lower dietary levels.

The remaining findings, considered incidental and unrelated to treatment, included red nasal stain in one control male (PND 35 and PND 45) and a dermal lesion described as a scab in one low- and high-dose male, each and two mid-dose males all occurring on PND 60. There were no incidental findings in females at any dietary level.

b. Motor activity:

Two control animals were inadvertently tested in the same figure-eight maze on PND 13 and PND 17. This inadvertent error did not adversely impact the outcome of the test. For males, one low-, mid- and high-dose animal, each were not tested on PND 120 because they were used for PND 70 brain weight measurements. Also, one low-dose male was found dead on PND 56 and, therefore, was not tested on PND 60 or 120. For females, one low- and mid-dose animal, each and two high-dose animals were not tested on PND 120 because they were scheduled for PND 70 brain weight determinations. Also, one control female was found dead on PND 86 and, therefore, was not tested on PND 120. The remaining sample size at the low- (14 or 15 males and 15 females), mid- (15 males and females, each) and high- (15 males and 14 females) dose was considered sufficient to establish whether there were compound-related effects.

Summary Session Motor and Locomotor Activity

Performance for Controls. Age-related changes in the levels of motor and locomotor activity were evident in control males and females. The greatest difference in activity for the overall 60-minute test session was apparent as very low levels of locomotor activity in the youngest animals (PND 13), compared to subsequent test occasions. This outcome is consistent with their relatively undeveloped ambulatory skills and sensory function (e.g., eyelids closed). This was followed by a progressive increase in levels of motor and locomotor activity with age. Gender-related differences in activity were apparent on PND 60 and 120 only, with higher levels of motor and locomotor activity for females, compared with males. These comparisons (within the control group) describing performance by age and gender were not subjected to statistical analysis.

There were no compound-related effects on measures of motor or locomotor activity in males or females at any dietary level. Moreover, there were no statistical differences from control at any dose level on any test occasion.

Table 5.7.1-16: Mean (±S.D.) Motor Activity Data (total activity counts for session)

Test Day	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Males				
PND 13	71±56 (16)	92±112 (16)	101±79 (16)	65±67 (16)
PND 17	180±91 (16)	185±132 (16)	222±98 (16)	200±106 (16)
PND 21	301±136 (16)	261±142 (16)	289±58 (16)	208±141 (16)
PND 60	511±102 (16)	557±106 (15)	538±119 (16)	543±109 (16)
PND 120	383±66 (16)	401±115 (14)	425±142 (15)	398±98 (15)
Females				
PND 13	42±34 (16)	40±33 (16)	81±65 (16)	61±47 (16)
PND 17	213±148 (16)	167±99 (16)	192±126 (16)	170±114 (16)
PND 21	312±108 (16)	300±110 (16)	263±78 (16)	260±106 (16)
PND 60	696±108 (16)	695±253 (16)	710±169 (16)	730±185 (16)
PND 120	542±131 (15)	546±193 (15)	507±167 (15)	580±229 (14)

Values are mean ± S.D. (*n*) Values were not statistically different from control, $p \leq 0.05$

Table 5.7.1-17: Mean (±S.D.) Locomotor Activity Data (total activity counts for session)

Test Day	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Males				
PND 13	8±9 (16)	15±22 (16)	10±10 (16)	10±13 (16)
PND 17	45±27 (16)	47±35 (16)	55±28 (16)	50±32 (16)
PND 21	93±36 (16)	73±27 (16)	92±28 (16)	75±38 (16)
PND 60	344±71 (16)	384±101 (15)	381±102 (16)	383±89 (16)
PND 120	255±56 (16)	257±72 (14)	281±99 (15)	270±61 (15)

Test Day	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Females				
PND 13	7±10 (16)	4±6 (16)	9±14 (16)	7±10 (16)
PND 17	57±49 (16)	50±38 (16)	50±34 (16)	46±36 (16)
PND 21	91±38 (16)	84±31 (16)	79±33 (16)	80±36 (16)
PND 60	471±112 (16)	438±164 (16)	456±132 (16)	482±185 (16)
PND 120	350±90 (15)	369±179 (15)	335±110 (15)	373±156 (14)

Interval Motor and Locomotor Activity:
Performance for Controls. Motor and locomotor activity data were also subjected to analysis at each 10-minute interval of the 60-minute test session. Evaluation of the progressive decrease in activity over the course of a test session provides a measure of habituation. For motor activity, habituation was evident in both sexes at all five ages, including PND 13, when activity levels were relatively low. For locomotor activity, habituation was apparent in controls at all ages except PND 13, when activity was so low (an average of 2 counts, each) during the first interval for males and females that habituation was not evident.
A comparison of interval results for control and treated animals revealed no compound-related effects at any dietary level. Levels of motor and locomotor activity were generally comparable to control for all test intervals on all test occasions. Moreover, there were no statistical differences from control in males or females at any dietary level on any test occasion.

c. Auditory startle habituation

One mid-dose male was inadvertently tested using the same load cell on PND 22 and PND 60. This inadvertent error did not adversely affect the outcome of the test.
Performance for Controls. The amplitude of the startle response increased with age in both sexes. This reflects a true age-related increase in the force of the response, since body weight is not included in the measure of response amplitude. The average response amplitude on PND 22 and 60 (+2 days) was 28 and 157 g, respectively, for control males, and 27 and 73 g, respectively, for control females. Habituation was apparent in control males and females as a decrease in response amplitude over the course of the test session, except in control males on PND 22 when response amplitude was relatively low throughout the test session. These comparisons (within the control group) to describe performance by age were not subjected to statistical analysis.
Startle amplitude, latency and habituation were not affected by treatment at any dietary level, on any test occasion. Furthermore, there were no statistical differences from control at any dietary level on either test occasion.

Table 5.7.1-18. Auditory Startle Reflex Peak Amplitude Data (g, mean ± S.D.)

Block		Dietary Level of Deltamethrin			
		Control	20 ppm	80 ppm	200 ppm
Males					
PND 23	Block 1	28±9	26±11	29±14	27±12
	Block 2	29±10	27±11	27±13	28±10
	Block 3	28±9	25±11	28±14	28±13
	Block 4	27±12	23±12	27±10	25±12
	Block 5	26±9	19±9	24±10	22±9
	Avg. For Total Session	**28±9**	**24±10**	**27±11**	**26±10**
	No. Of Animals	16	16	16	16
	Body Weight	52	53	52	48
PND 60	Block 1	175±129	173±72	171±109	164±77
	Block 2	183±153	138±107	189±142	186±106
	Block 3	198±156	124±96	155±113	167±84
	Block 4	142±117	115±83	120±92	126±55
	Block 5	116±99	111±57	127±95	91±40
	Avg. For Total Session	**157±128**	**112±79**	**148±108**	**143±63**
	No. Of Animals	16	16	16	16
	Body Weight	273	280	270	272
Females					
PND 23	Block 1	32±15	29±10	28±19	33±14
	Block 2	29±15	28±9	30±15	30±13
	Block 3	27±13	30±10	27±13	26±8
	Block 4	26±10	26±10	26±12	26±8
	Block 5	22±11	23±8	25±13	21±6
	Avg. For Total Session	**27±12**	**27±8**	**27±13**	**27±8**
	No. Of Animals	16	16	16	16
	Body Weight	52	51	48	48
PND 60	Block 1	86±53	92±41	120±68	114±65
	Block 2	70±49	98±68	129±74	102±66
	Block 3	70±37	93±69	111±59	95±64
	Block 4	71±49	61±39	85±49	77±52
	Block 5	59±30	54±31	64±35	71±56
	Avg. For Total Session	**73±37**	**80±42**	**102±52**	**92±56**
	No. Of Animals	16	16	16	16
	Body Weight	174	179	168	171

Values are mean ± S.D.

d. Learning and memory testing:

Postweaning – Passive avoidance:

For acquisition and retention, there was no evidence of a compound-related effect in males or females at any dietary level. Moreover, there were no statistical differences from control at any dietary level in either sex.

Table 5.7.1-19: Passive Avoidance Performance at PND 22 and 29 (mean ± S.D.)

Session/Parameter		Dietary Level of Deltamethrin			
		Control	20 ppm	80 ppm	200 ppm
Males					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.9±0.3	3.2±0.8	3.1±0.6	2.9±0.3
	Latency trial 1 (seconds)	52.9±50.0	31.8±29.2	49.7±50.9	47.0±42.3
	Latency trial 2 (seconds)	180.0±0.0	169.6±41.7	180.0±0.0	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	1 (6%)	0 (0%)	1 (6%)	1 (6%)
Session 2 (Retention Phase)	Number of Animals Tested	15	16	15	15
	Number of Animals Included in Analysis	15	16	15	15
	Trials to criterion	2.3±0.7	2.4±0.8	2.3±0.7	2.2±0.4
	Latency trial 1 (seconds)	180.0±0.0	177.8±8.7	178.4±6.1	160.4±50.8
	Latency trial 2 (seconds)	169.2±29.7	166.8±40.3	173.6±17.0	180.0±0.0
Females					
Session 1 (Learning Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.0±0.0	3.3±1.0	3.2±0.4	3.3±0.7
	Latency trial 1 (seconds)	25.5±24.7	38.7±39.2	47.4±42.1	23.5±17.2
	Latency trial 2 (seconds)	180.0±0.0	180.0±0.0	172.4±21.3	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	1 (6%)	0 (0%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.3±0.6	2.3±0.5	2.1±0.3	2.4±0.7
	Latency trial 1 (seconds)	162.6±47.7	151.6±50.7	175.9±13.2	166.2±34.2
	Latency trial 2 (seconds)	171.0±36.0	180.0±0.0	180.0±0.0	174.2±19.8

Trials to Criterion = Mean No. Trials per Group ± S.D.
Latency to Trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion = Number of animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of animals that never received the shock.

Adult Offspring – Water maze:

There were no compound-related effects in males or females at any dietary level. There was a statistical difference from control during acquisition in mid- and high-dose females involving a significant decrease in the number of errors during the first trial (0.6 and 0.4, respectively vs. an average 1.6 errors for controls). This was considered incidental and unrelated to treatment because this

difference from control was not consistent between sexes and the results at both the mid- and high-dose are within the range of historical controls (average 0.3 to 1.3 errors), whereas the number of errors for controls was above the range for historical control.

Table 5.7.1-20: Water Maze Performance on PND 60 and Seven Days Later (mean ± S.D.)

Session/Parameter		Dietary Level of Deltamethrin			
		Control	20 ppm	80 ppm	200 ppm
Males					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	6.9±2.4	6.8±2.4	6.5±2.0	7.9±2.1
	Trial 1 - Errors (Mean±S.D.)	0.6±0.9	1.0±1.2	0.6±0.6	0.8±0.9
	Trial 1 - Duration (seconds) (Mean±S.D.)	18.8±15.1	21.9±15.0	12.1±5.9	19.3±18.4
	Trial 2 - Errors (Mean±S.D.)	0.6±0.6	0.3±0.7	0.6±1.0	0.9±0.8
	Trial 2 - Duration (seconds) (Mean±S.D.)	12.6±9.9	17.6±15.6	16.0±15.9	20.3±15.1
	Failed to Meet Criterion	1 (6%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (Retention Phase)	Number of Animals	15	16	16	16
	Trials to Criterion (Mean±S.D.)	5.2±0.4	6.0±1.6	5.6±1.7	5.6±1.1
	Trial 1 - Errors (Mean±S.D.)	0.2±0.4	0.4±0.9	0.4±0.7	0.6±1.5
	Trial 1 - Duration (seconds) (Mean±S.D.)	7.5±4.3	10.6±11.4	8.5±7.2	11.2±14.7
	Trial 2 - Errors (Mean±S.D.)	0.0±0.0	0.0±0.0	0.2±0.8	0.1±0.3
	Trial 2 - Duration (seconds) (Mean±S.D.)	4.7±1.5	4.3±1.3	5.3±6.2	4.2±1.6
Females					
Session 1 (Learning Phase)	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	8.1±2.1	7.4±2.3	7.2±2.0	8.1±2.8
	Trial 1 - Errors (Mean±S.D.)	1.6±1.5	0.8±0.9	*0.6±0.6	*0.4±0.9
	Trial 1 - Duration (seconds) (Mean±S.D.)	23.3±17.0	16.6±9.3	12.8±6.9	13.9±13.4
	Trial 2 - Errors (Mean±S.D.)	1.0±1.2	0.6±0.7	0.9±1.2	0.6±0.7
	Trial 2 - Duration (seconds) (Mean±S.D.)	17.9±15.0	11.8±5.2	14.0±14.2	10.4±5.3
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Session 2 (Retention Phase)	Number of Animals	16	16	16	15
	Trials to Criterion (Mean±S.D.)	7.6±3.2	6.5±2.7	7.2±3.6	8.7±3.5
	Trial 1 - Errors (Mean±S.D.)	0.6±0.8	0.4±1.3	0.3±0.6	0.1±0.4
	Trial 1 - Duration (seconds) (Mean±S.D.)	9.9±7.2	9.5±11.1	6.6±4.4	6.3±3.7
	Trial 2 - Errors (Mean±S.D.)	0.3±0.6	0.3±0.7	0.1±0.3	0.7±1.0
	Trial 2 - Duration (seconds) (Mean±S.D.)	5.3±3.8	5.2±3.3	4.1±1.7	7.6±5.2

Values for rats who failed to learn during Session 1 were not included in means for Session 2.
Values are mean ± standard deviation * Statistically different from control, p_0.05

e. Ophthalmology

There were no test substance-related lesions in males or females at any dietary level.

f. Postmortem results:

1. Gross Pathology:

There were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination.

2. Terminal Body Weight:

Day 21 - Terminal body weight for male and female pups was not significantly different from control in males or females at any dietary level.
Terminal - Terminal body weight for perfused and non-perfused males and females was not affected by treatment at any dietary level.

3. Brain Weights:

Day 21 - Absolute and relative fixed brain weights were not affected by treatment in males or females at any dietary level.
Terminal - Absolute and relative fixed brain weights were not affected by treatment in males or females at any dietary level. The only difference from control was a statistically-reduced absolute fixed brain weight for high-dose perfused females. This difference from control is not considered a compound-related effect, since there was no similar difference in non-perfused high-dose females nor in perfused or non-perfused high-dose males. In addition, there was no difference in relative brain weight in males or females, perfused or non-perfused, at any dietary level.

Table 5.7.1-21: Mean (± S.D.) Brain Weight Data

Parameter	Dietary Level of Deltamethrin			
	Control	20 ppm	80 ppm	200 ppm
Males				
PND 21(Perfused)				
Terminal Body Weight (g)	48.3±6.5 (10)	49.3±3.7 (10)	48.9±6.1 (10)	44.8±2.9 (10)
Brain, Fixed (g)	1.416±0.065 (10)	1.396±0.053 (10)	1.412±0.064 (10)	1.415±0.047 (10)
Brain, Fixed/Body Weight (%)	2.978±0.386 (10)	2.845±0.192 (10)	2.923±0.310 (10)	3.163±0.155 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	326.3±22.2 (10)	323.2±16.6 (10)	320.5±31.3 (10)	318.6±19.8 (10)
Brain, Fixed (g)	1.862±0.065 (10)	1.867±0.117 (10)	1.874±0.067 (10)	1.891±0.108 (10)
Brain, Fixed/Body Weight (%)	0.573±0.043 (10)	0.578±0.024 (10)	0.589±0.052 (10)	0.595±0.051 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	320.5±24.1 (10)	328.7±16.5 (10)	316.6±26.6 (10)	325.9±14.3 (10)
Brain, Fresh (g)	1.932±0.082 (10)	1.935±0.129 (10)	1.883±0.108 (10)	1.945±0.091 (10)
Brain, Fresh/Body Weight (%)	0.605±0.040 (10)	0.589±0.035 (10)	0.598±0.059 (10)	0.598±0.036 (10)
Females				
PND 21(Perfused)				
Terminal Body Weight (g)	48.9±3.4 (10)	45.2±5.3 (10)	45.3±5.0 (10)	45.2±4.5 (10)
Brain, Fixed (g)	1.390±0.046 (10)	1.352±0.048 (10)	1.363±0.055 (10)	1.346±0.072 (10)
Brain, Fixed/Body Weight (%)	2.860±0.254 (10)	3.026±0.355 (10)	3.039±0.307 (10)	2.994±0.229 (10)
PND 75 (±5) (Termination - Perfused)				
Terminal Body Weight (g)	193.9±13.1 (9)	195.0±11.6 (10)	192.6±11.4 (10)	189.1±12.2 (10)
Brain, Fixed (g)	1.793±0.073 (10)	1.785±0.076 (10)	1.753±0.064 (10)	1.673*±0.088 (10)
Brain, Fixed/Body Weight (%)	0.928±0.078 (9)	0.919±0.069 (10)	0.913±0.066 (10)	0.887±0.062 (10)
PND 75 (±5) (Termination - Non-Perfused)				
Terminal Body Weight (g)	200.00±16.4 (10)	199.0±15.9 (10)	188.5±9.8 (10)	191.4±14.6 (10)
Brain, Fresh (g)	1.820±0.077 (10)	1.772±0.120 (10)	1.766±0.087 (10)	1.764±0.099 (10)
Brain, Fresh/Body Weight (%)	0.915±0.085 (10)	0.892±0.038 (10)	0.938±0.055 (10)	0.925±0.070 (10)

* Statistically different from control, $p \leq 0.05$ Values are mean ± S.D. (n)

4. Gross brain measurements:

Day 21 Pup Gross Brain Measurements: For perfused day 21 pups, the cerebrum and cerebellum lengths were not significantly different from control in males or females at any dietary level.
Terminal Animal Gross Brain Measurements: For perfused terminal adults, the cerebrum and cerebellum lengths were comparable to control for males and females at all dietary levels.

Day 21 Pup Micropathology Brain Measurements: There were no compound-related effects on any brain measurement in high-dose males or females.
Terminal Animal Micropathology Brain Measurements: There were no compound-related effects on any brain measurement in high-dose males or females. There was a statistically significant increase in the hippocampus thickness at the highest dietary level in males only (12% more than control). Since the value for high-dose males was within the range of historical control (unlike the control value) and there was no corresponding finding in females this was not considered related to treatment. There were no other findings attributed to treatment in males or females at any dietary level.

Table 5.7.1-22: Histology findings

PND 75 (±5) (*Termination - Perfused)*	**Males**		**Females**	
Brain Anatomical Area	**Deltamethrin (ppm)**		**Deltamethrin (ppm)**	
	0	**200**	**0**	**200**
Hippocampal Gyrus	1.5543±0.0324 (10)	1.7255*±0.0156 (10)	1.6036±0.0359 (10)	1.6345±0.0139 (10)

* Statistically different from control, p>0.05

5. Micropathology:

Day 21 Pup Micropathology: There were no compound-related microscopic findings in brain tissues from perfused PND 21 high-dose males or females.
Terminal Animal Micropathology: There were no compound-related microscopic findings in brain tissues from perfused terminal high-dose males or females.

Additional Non-Brain Terminal Animal Tissues: Spinal cord (cervical, thoracic, and lumbar), cauda equina, spinal nerve roots and dorsal root ganglia (cervical and lumbar), gasserian ganglion, eyes, optic nerves, gastrocnemius muscle, sciatic nerve, tibial nerve, and sural nerves were also evaluated microscopically from perfused terminal animals. There were no compound-related microscopic lesions present in any tissue from the perfused high-dose terminal males or females.

III. Conclusions

Technical-grade deltamethrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats, at nominal concentrations of 0, 20, 80 and 200 ppm.The average daily intake of active ingredient during gestation and lactation was 0, 1.64, 6.78 and 16.1 mg/kg/day. There was no effect on reproduction parameters at any dietary level.

Maternal
Compound-related effects consisted of the following:
20 ppm None (NOAEL)
80 ppm None (NOAEL)
200 ppm Significantly decreased body weight, weight gain and food consumption during gestation and lactation.

Offspring
Compound-related effects were limited to the following:
20 ppm None (NOAEL)
80 ppm None (NOAEL)
200 ppm Significantly decreased pre- and post-weaning body weight and weight gain, vocalizations with handling in males on PND 4, and a delay in balanopreputial separation in correlation with body weight decrease.

CA 5.7.2 Delayed polyneuropathy studies

No new studies have been performed since the last EU review.

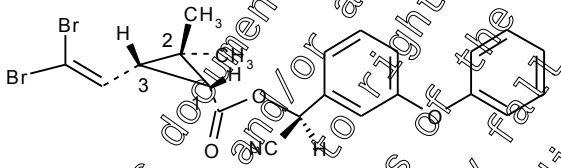
CA 5.8 Other toxicological studies

CA 5.8.1 Toxicity studies of metabolites

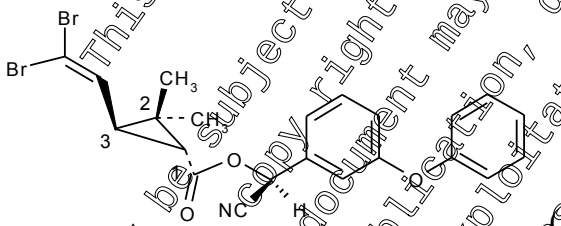
In the last EU review, an acute oral rat, an Ames test and *in vivo* mouse micronucleus test performed on the major soil and water metabolite, becisthemic acid (Br_2CA), were submitted. In this dossier new toxicological studies on the trans isomer of deltamethrin are summarized. A copy of the summaries performed by the former RMS Sweden, available in the Monograph 1998 or its addendum Rev2 July 2002 is also available thereafter.
Becisthemic acid Technical was administered as a suspension in arachis oil BP to male Sprague-Dawley rats at 500, 1000 and 2000 mg/kg b.w. In order to determine the relative sensitivity of the untreated sex, an additional group of five female animals was treated at 2000 mg/kg.
Mortality was observed at 2000 mg/ml in male rats only. Two males were found dead 2 and 4 hours after dosing, and two others died one day after dosing. Ataxia, hunched posture, lethargy and decreased respiratory rate were observed in all dose groups. At 2000 mg/ml diarrhoea, diuresis, ptosis and laboured respiration were observed in both males and females. Loss of righting reflex was also noted in males from this group. Most surviving animals recovered one or two days after dosing. Haemorrhagic lungs, dark liver, dark kidneys, slight haemorrhage of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhage of the small and large intestines were observed at necropsies of the dead animals. No abnormalities were noted at necropsy of animals killed at the end of the study. The acute median lethal dose (LD_{50}) of becisthemic acid was calculated to be 1682 mg/kg b.w (95% confidence limits were 1091 to 2594 mg/kg b.w) in males and greater than 2000 mg/kg b.w. in females.

Technical becisthemic acid (Batch 7 N 0589 B; 99.9% purity) was tested for its ability to induce mutation in 4 strains of *Salmonella Typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 uvrA-). Becisthemic acid was found to be negative in the Ames test.
Technical becisthemic acid (Batch 7 N 0589 B; 99.9% purity) was tested for its ability to induce micronuclei in polychromatic and normochromatic erythrocytes of Crl:CD-1 (ICR) BR Strain mice. In the main study, groups of mice (5/sex/group) were dosed once only by gavage at 500, 1000 or 2000 mg/kg bw and killed 24 hours following treatment. A second group (5/sex) dosed at 2000 mg/kg bw was killed 48 hours post dosing. Control groups (5/sex/group) included 2 groups treated with PEG 200 killed at 24 or 48 hours post dosing and one positive control group treated with cyclophosphamide and killed 24 hours post dosing.
Becisthemic acid did not induce micronuclei in the polychromatic erythrocytes in the bone marrow of mice treated up to 2000 mg/kg bw.
As Becisthemic acid is also a major rat metabolite (under unconjugated and conjugated forms) found well above 10% of the administered dose in the rat metabolism study, no new toxicological studies have been performed since the last EU review.

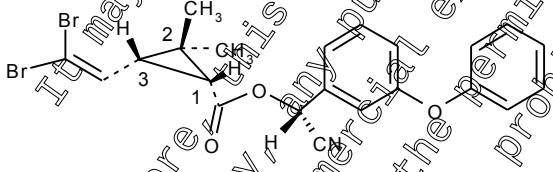
Among the eight possible isomeric forms of deltamethrin, only 2 of them were found in plant metabolism studies: the alpha-R (AE F108569) isomer (1*R*, 3*R*, α *R*) and the (1*R*, 3*S*, α*S*) *trans*-isomer of deltamethrin (AE 0035073). The formation of the former occurs by epimerisation of the parent isomer. The formation of the (1*R*, 3*S*, α*S*) *trans*-isomer of deltamethrin can be explained by the photoisomerisation of parent (1*R*, 3*R*, α*S*) deltamethrin.



(1*R*, 3*R*, α*S*) *cis* deltamethrin = deltamethrin



(1*R*, 3*S*, α*S*) *trans*-isomer of deltamethrin



(1*R*, 3*R*, α*R*) alpha-R-isomer of deltamethrin

Bayer CropScience has developed a new LC-MS/MS method of analysis which quantifies individually the 3 isomers in the different commodities. Since 2009, all the supervised residue trials were analyzed with this new method. A significant set of residue data on the 3 aforementioned isomers is therefore available. One of the main trends identified in this database is that, at harvest, the alpha-R- isomer was not seen above the LOQ (0,01 or 0,05 mg/kg) and in the vast majority of the cases, it was reported below the LOD . Therefore the alpha-R-isomer, may be virtually disregarded in this respect. As a consequence, we have only taken into account the *trans*-isomer as relevant compound for further

consideration. To assess the possible health risk due to potential dietary exposure to the *trans*-isomer, a new conservative approach has been applied on the basis of the Scientific Opinion on Evaluation of the toxicological relevance of pesticide metabolites for dietary risk assessment (EFSA Journal 2012 ; 10(07) : 2799). This Scientific Opinion refers to a decision tree which allows the evaluation of the human safety on the basis of the exposure to a metabolite.
According to this approach (referred as the Threshold of Toxicological Concern (TTC) approach), if the exposure of the metabolite is above 0.0025 µg/kg bw/day, *in vitro* genotoxicity studies are needed to demonstrate the non genotoxic potential of the metabolite; if the chronic exposure is below 1.5 µg/kg bw/day and the acute exposure is below 5 µg/kg bw/day, the metabolite is considered neither relevant nor further testing is required.
Bayer CropScience conducted the TTC concept approach in order to evaluate the relevance of the *trans*-isomer. A detailed assessment is presented in the document referenced:

Report:	KCA 5.8.1/04; [redacted]; 2013; M-448284-01-1
Title:	Estimation of trans-isomer of deltamethrin exposure - Applicability of the TTC concept
Report No.:	M-448284-01-1
Document No.:	M-448284-01-1
Guideline(s):	not specified
Guideline deviation(s):	not specified
GLP/GEP:	**no**

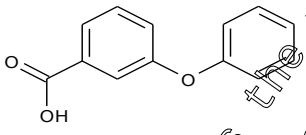
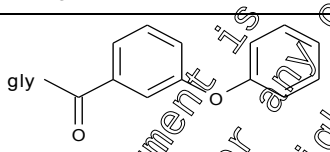
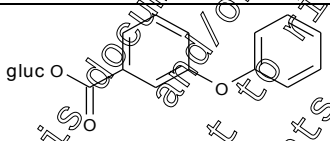
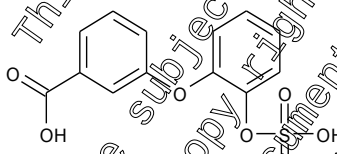
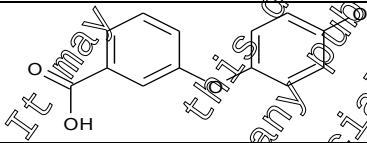
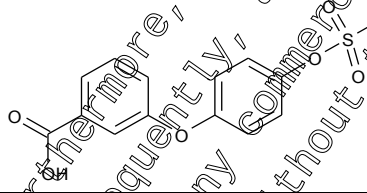
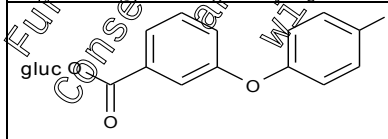
The summary of the trials is also available in dRR format:

Report:	KCA 5.8.1/09; [redacted]; 2016; M-559648-01-1
Title:	Compilation of dRR tables for deltamethrin residue studies from 2009 onwards - Results displayed for cis-deltamethrin, trans isomer and alpha-R isomer
Report No.:	M-559648-01-1
Document No.:	M-559648-01-1
Guideline(s):	none
Guideline deviation(s):	none
GLP/GEP:	**no**

As demonstrated, the chronic exposure to the *trans*-isomer according to EFSA PriMo model rev.2 can be estimated to **0.6 µg/kg bw/d** and the maximum estimated acute exposure for the *trans*-isomer can be calculated as **3.23 µg/kg bw/d**. The maximum estimated acute exposure and the chronic exposure to the *trans*-isomer are far above the limit of 0.0025 µg/kg/day. This means that the non genotoxic potential of the *trans*-isomer needs to be demonstrated in *in vitro* genotoxicity studies. However both exposures are below the limits established for Cramer class III compounds to which the pyrethroid class belong (1.5 µg/kg bw/day for chronic exposure and 5 µg/kg bw/day for acute exposure). Therefore no toxicity testing after repeat administration on the *trans*-isomer is needed. As summarized in this paragraph, the trans isomer of deltamethrin has no genotoxic potential in the Ames test, the HPRT test and in the *in vitro* chromosome aberration test. The acute oral rat demonstrated that the trans isomer is slightly less toxic than deltamethrin but with also a neurotoxic profile.

Although deltamethrin proved to be stable under pasteurisation and baking/brewing/boiling processes, results of the sterilisation process (120 °C, pH 6, 20 min) showed that deltamethrin was degraded to 3-phenoxybenzylaldehyde and (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (Br_2CA). These two substances were identified also as plant metabolites. Br_2CA has been identified also in rat metabolism and is considered of lower toxicity than parent compound. Regarding 3-phenoxybenzaldehyde, no toxicological data is available but it is a common metabolite of several pyrethroids. As explained in a recent position paper ([redacted]; 2013; M-466413-01-1, see KCA 6.5.1/02), this aldehyde is a transient molecule and therefore was not isolated in the metabolism study but can be considered to have been present as an intermediate. Indeed, the results of a rat metabolism study ([redacted] 1978; M-063782-01-1) indicate the rates of 3-phenoxybenzaldehyde-related metabolites account for 77.1 % of the applied dose. Please refer to the table below.

Table 5.8.1-01: Structure and occurrence of these metabolites

Structure	Name (IUPAC)	% of administered dose in the rat
	3-phenoxybenzoic acid	4.5%
	*N*-(3-phenoxybenzoyl)glycine	3.6%
	1-*O*-(3-phenoxybenzoyl)-L-glucopyranuronic acid	12.6%
	3-[2-(sulfooxy)phenoxy] benzoic acid	2.0%
	3-(4-hydroxyphenoxy)benzoic acid	4.0%
	3-[4-(sulfooxy)phenoxy]benzoic acid	48.6%
	1-*O*-[3-(4-hydroxyphenoxy)benzoyl]-L-glucopyranuronic acid	1.8%
TOTAL		**77.1%**

It can be therefore concluded that the toxicological effects of 3-phenoxybenzaldehyde were intensively co-tested in toxicity studies where animals were administered with deltamethrin. No further toxicological studies are therefore needed.

Table 5.8.1-02: Summary of toxicity studies with deltamethrin metabolites

Study	Species	Results
-Becisthemic acid (Br_2CA)		
[redacted] test M-152479-01-1	*Salmonella Typh. and Escherichia coli*	Negative
In vivo mouse micronucleus test M-152481-01-1	CD-1 mice	Negative
Rat Acute Oral Study M-152480-01-1	Sprague Dawley Rat	LD_{50} = 1682 mg/kg b.w in males LD_{50} > 2000 mg/kg b.w in females
-Trans isomer		
Rat Acute Oral Study **M-461316-01-1**	Female Sprague Dawley	LD_{50} above but close to 178 mg/kg
Ames test **M-228638-01-1**	Salmonella Typh.	Negative
Gene mutation assay (HPRT) **M-461312-01-1**	Chinese Hamster V79 cells	Negative
Chromosome aberration test **M-469011-01-1**	Chinese Hamster V79 cells	Negative

Additional information on metabolites provided on request of the RMS can be found in document M-559823-01-1.

In addition to the toxicological studies on the becisthemic acid (Br_2CA) metabolite already available in the Monograph and baseline dossier, additional toxicological studies were performed on the trans isomer of deltamethrin. They are summarized below.

Becisthemic acid (Br_2CA) metabolite:

Bacterial reverse mutation assay ("Ames test")

Report: KCA 5.8.1/01; [redacted]; 1997; M-152479-01-1
Title: Bacterial reverse mutation assay (Ames Test) Becisthemic acid Code: RU23441
Report No.: A74229
Document No.: M-152479-01-1
Guideline(s): EU (=EEC): ; OECD: ; USEPA (=EPA):
Guideline deviation(s): --
GLP/GEP: **yes**

Experimental design

cis-Br_2CA (purity 99.9%) was evaluated for mutagenic activity in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100 and *Escherichia coli* strain WP2uvrA with and without metabolic activation (rat liver S9-mix) using the Ames plate incorporation method. The dose levels were 0, 5, 50, 150, 500, 1500 and 5000 µg/plate. The solvent used was dimethyl sulphoxide (DMSO). Positive controls were N-ethyl- N´-nitro-N-nitrosoguanidine (ENNG), 9-aminoacridine (9AA), 4-nitroquinoline-1-oxide (4NQO) and 2-aminoanthracene (2AA). The experiment was repeated on a separate day using the same methodology and dose levels as for the first experiment.

Results

The test material caused a visible reduction in the growth of the bacterial lawn to all of the tester strains, both with and without metabolic activation at 1500 µg/plate and above. Thus, it was toxic to the tester strains. No significant increases in the frequency of revertant colonies were recorded for any of the bacterial strains, with any dose of the test material, either with or without metabolic activation. All of the positive control chemicals used in the test induced marked increases in the frequency of revertant colonies.

Comments

Under the test conditions used in this study, Br_2CA was not found to be mutagenic in *Salmonella typhimurium* strains TA1535,TA1537,TA98,TA100 or *Escherichia coli* strain WP2uvrA in the presence or absence of metabolic activation. The study followed OECD guideline no 471. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Micronucleus test in the mouse

Report:	KCA 5.8.1/02; [redacted], 1997; M-152481-01-1
Title:	Mouse micronucleus test Becisthemic acid Code: RU23441
Report No.:	A74231
Document No.:	M-152481-01-1
Guideline(s):	EU (=EEC): 84/449/EEC B12; OECD: 474
Guideline deviation(s):	-
GLP/GEP:	**yes**

Experimental design

cis-Br_2CA (purity 99.9%) was dissolved in polyethylene glycol (PEG 200) and administered orally as a single dose to 8- week old mice ([redacted]) at levels of 500, 1000 and 2000 mg/kg bw. Each group consisted of 5 animals/sex. Mice in a second group were dosed orally with the test substance at the dose level of 2000 mg/kg bw. The animals were killed at 24 or 48 h after dosing and the frequencies of micronuclei in bone marrow normochromatic and polychromatic erythrocytes were determined. The positive controls received cyclophosphamide at 50 mg/kg bw. The negative controls (consisted of 2 groups of animals) received PEG 200 only.

Results

One female animal died within 24 h after treatment with the test substance at the dose level of 2000 mg/kg bw. Clinical signs (hunched posture, lethargy, decreased respiratory rate, labourated respiration, ptosis, ataxia, splayed gait and body tremors) were at sample time observed in some of the animals dosed with the test substance at the dose level of 2000 mg/kg bw. No statistically significant increase in the frequency of micronucleated normochromatic or polychromatic erythrocytes was observed in animals dosed with the test substance when compared to the negative controls. No statistically significant decreases in the ratio of polychromatic erythrocytes were observed at any sample time. The positive controls showed a significant increase in the frequency of micronucleated polychromatic erythrocytes.

Comments

Under the test condition used in this study Br_2C did not produce micronuclei in the polychromatic erythrocytes in the mouse. The study follows OECD guideline no. 474 except for some minor deviations. The humidity in the experimental animal room varied between 70-75% recommended values according to the OECD guideline no 474 are 30-70%. According to the guideline no 474 (adopted 21st July 1997) it is not necessary to score normochromatic erythrocytes for incidence of micronuclei when animals are treated only once. When normochromatic erythrocytes are scored for incidence of micronuclei samples of peripheral blood should be analysed. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Acute oral rat

Report:	KCA 5.8.1/03, [redacted], 1997; M-152480-01-1
Title:	Rat acute oral toxicity study Becisthemic acid Code: RU 23441
Report No.:	A74230
Document No.:	M-152480-01-1
Guideline(s):	EU (=EEC): 92/69/EEC; OECD: 401
Guideline deviation(s):	--
GLP/GEP:	**yes**

Experimental design

cis-Br_2C (purity 99 %) was dissolved in arachis oil BP (peanut oil) and administered orally by gavage as a single dose to fasted male rats at the dose levels of 500, 1000 and 2000 mg/kg bw. Each group consisted of five male rats (Sprague-Dawley [redacted]). Additionally, five fasted female rats (Sprague-Dawley [redacted]) were similarly treated at a dose level of 2000 mg/kg bw. The observation period was 14 days. All animals were subjected to gross pathological examination.

Results

LD_{50} was estimated at 1682 mg/kg bw/day for fasted male rats (95% confidence limits were 1091-2594 mg/kg bw). LD_{50} for fasted female rats was calculated to be greater than 2000 mg/kg bw. There was no mortality for females in this study. Clinical signs of systemic toxicity noted in both sexes were ataxia, diarrhoea, diuresis, hunched posture, lethargy, ptosis, decreased respiratory rate,

laboured respiration, loss of righting reflex, red/brown staining around the mouth or snout and splayed gait. Surviving animals recovered one or two days after dosing except for one male treated with the test material at the dose level of 1000 mg/kg bw which showed red/brown staining around the eyes five days after dosing and at subsequent observation, and one male treated with the test material at the dose level of 500 mg/kg bw which showed similar red/brown staining on day 8. Surviving animals showed expected gain in bodyweight during the study. Gross examination of the animals that died showed haemorrhagic lungs, dark liver, dark kidneys, slight haemorrhage of the gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrage of the small and large intestines. No gross pathological changes were observed in animals necropsied 14 days after exposure to the test material.

Comments

The study follows OECD guideline no 401. The study was conducted in accordance with the principles of GLP and subjected to Quality Assurance inspections. The study seems to be of acceptable quality.

Trans isomer of deltamethrin:

Report:	KCA 5.8.1/05; [redacted]; 2013; M-461316-01-1
Title:	Trans isomer of deltamethrin - Acute oral toxicity study in rats
Report No.:	13/055-001P
Document No.:	M-461316-01-1
Guideline(s):	This study was based on the principles of the following guidelines, but the dose levels used and group sizes were designed to meet a specific Sponsor requirement; OECD Guidelines for Testing of Chemicals No. 423(Acute Oral Toxicity - Acute Toxic Class Method, adopted: 17th December 2001); EEC Directive 440/2008, B.1.tris (O.J. L142); EPA Health Effects Test Guidelines (OPPTS 870.1100), United States, EPA 712-C-98-190 (1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material: Trans isomer of Deltamethrin

- Article no.: AE 0035073
- Description: White solid
- Lot/Batch no: SES 10538-2-2
- Purity: 94.5%
- Stability of test compound: guaranteed for study duration; expiry date: 2014-03-01

2. Vehicle: Corn oil

3. Test animals:

- Species: Rat
- Strain: Sprague Dawley

Age:	9 weeks old
Weight at dosing:	201 g – 236 g
Source:	[redacted] Germany, [redacted]
Acclimatisation period:	at least 5 days
Diet:	[redacted] Autoclavable complete diet for rats and mice – breeding and maintenance [redacted], Germany, *ad libitum*
Water:	tap water, ad libitum
Housing:	3 animals per cage in Type II polypropylene/polycarbonate cages on Lignocel Bedding for Laboratory Animals
Environmental conditions:	Temperature: 22 ± 3°C Humidity: 50 ± 20% Air changes: Approximately 15-20 changes per hour Photoperiod: Alternating 12-hour light and dark cycles

B. Study Design and methods

1. In life dates

28 May to 14 June 2013.

2. Animal assignment and treatment

The single-dose oral toxicity of trans isomer of deltamethrin, AE 0035073 was investigated according to the general principles of the acute toxic class method (OECD 423), but to a custom study design, in Sprague Dawley rats. The dose levels of **75**, **100**, **133** and **178 mg/kg** body weight were tested in non-fasted Sprague Dawley female rats. A single oral treatment was administered by gavage to each animal. Trans isomer of deltamethrin, AE 0035073 was formulated in corn oil at a concentration of 15, 20, 26.6 or 35.6 mg/mL, at a dosing volume of 5 mL/kg bw.

All groups consisted of three females.

Initially, three females (Group 1) were treated at a dose level of 75 mg/kg bw. The test item did not cause mortality in this group; therefore the next dose group (Group 2) was treated at a dose level of 100 mg/kg bw. The test item did not cause mortality in this group; therefore the next dose group (Group 3) was treated at a dose level of 133 mg/kg bw. The test item did not cause mortality in this group; therefore the next dose group (Group 4) was treated at a dose level of 178 mg/kg bw. The test item did not cause mortality in this group but induced severe clinical signs consistent with a near-lethal dose; so no further testing was required according to the Sponsor requirement.

Clinical observations were performed at 30 minutes, 1, 2, 3, 4 and 6 hours after dosing and once daily for 14 days thereafter. Body weight was measured on Days -1, 0 (before treatment) and 7 and on Day 14 before necropsy. All animals were subjected to a necropsy and a macroscopic examination.

3. Statistics

The data did not warrant statistical analysis.

II. Results and discussion

A. Mortality

The trans isomer of deltamethrin, AE 0035073 did not cause any mortality at dose levels of 75, 100, 133 or 178 mg/kg bw.

B. Clinical observations

Clinical signs were observed in animals treated at 133 and 178 mg/kg bw with trans isomer of deltamethrin, AE 0035073.

Treatment with 133 mg/kg bw caused hunched back (3/3) and tremors (continuous) whole body (3/3). All animals became symptom-free from 6 hours after the treatment until the end of the observation period.

Treatment with 178 mg/kg bw caused hunched back (3/3), tremors (continuous) whole body (3/3) and clonic convulsion whole body (2/3). All animals were symptom-free from Day 1 after the treatment until the end of the observation period.

Table 5.8.1-03 : Clinical signs observed at 133 and 178 mg/kg bw treated groups

Dose Level	Animal Number	Observations	Observation days												Frequency
			0						1	2	3	4	5	6-14	
			30'	1h	2h	3h	4h	6h							
133	1572	Symptom free	+	+	+	-	-	+	+	+	+	+	+	+	18/20
		Hunched back	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
	1573	Symptom free	+	+	+	-	-	+	+	+	+	+	+	+	18/20
		Hunched back	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
	1574	Symptom free	+	+	+	-	-	+	+	+	+	+	+	+	18/20
		Hunched back	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
178	1575	Symptom free	+	+	+	-	-	-	+	+	+	+	+	+	17/20
		Hunched back	-	-	-	+	+	+	-	-	-	-	-	-	3/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
	1576	Symptom free	+	+	+	-	-	-	+	+	+	+	+	+	17/20
		Hunched back	-	-	-	+	+	+	-	-	-	-	-	-	3/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Clonic convulsion Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
	1577	Symptom free	+	+	+	-	-	-	+	+	+	+	+	+	17/20
		Hunched back	-	-	-	+	+	+	-	-	-	-	-	-	3/20
		Tremors(continuous) Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20
		Clonic convulsion Whole body	-	-	-	+	+	-	-	-	-	-	-	-	2/20

+: present -: absent
h: hour (s) ': minute
Frequence of observation : number of occurrence of observation/total number of observations

C. Body weight

Body weight and body weight gain of trans isomer of deltamethrin, AE 0035073 treated animals showed no indication of a treatment-related effect.

D. Necropsy

No test item-related macroscopic observations were seen in animals dosed at dose levels of 75, 100, 133 or 178 mg/kg bw and terminated on Day 14.
Dark/red discoloration of the thymus was seen in one female dosed at 75 mg/kg bw.

III. Conclusions

Under the conditions of this study, the acute oral LD50 value of the test item trans isomer of deltamethrin, AE 0035073 was found to be above, but relatively close to, 178 mg/kg bw in female Sprague Dawley rats.

In vitro genotoxicity – Bacterial assay for gene mutation

Report:	KCA 5.8.1/06; [redacted]; 2004; M-228638-01-1
Title:	AE 0035073 00 1B97 0001 - Salmonella microsome test - Plate incorporation and preincubation method
Report No.:	C040291
Document No.:	M-228638-01-1
Guideline(s):	EU (=EEC): 2000/32/EC, B13/14; OECD: 471; USEPA (=EPA): OPPTS 870.5100
Guideline deviation(s):	--
GLP/GEP:	**yes**

Executive Summary

In this *in vitro* assessment of the mutagenic potential of AE 0035073 00 1B97 0001, the trans isomer of deltamethrin (Batch 5E0551; 94.0% of purity), histidine dependent auxotrophic mutants of Salmonella typhimurium, strains TA 1535, TA 1537, TA 98, TA 100 and TA 102 were exposed to AE 0035073 00 1B97 0001 diluted in dimethyl sulphoxide (DMSO) at concentrations up to 5000 µg/plate. For each bacterial strain and dose level, 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 for each strain. After 48 hours of incubation at 37 °C, the numbers of revertant colonies were scored using an automated colony counter. Another assay testing a pre-incubation for 20 minutes at 37 °C was also performed at doses ranging from 16 to 5000 µg/plate.

Doses up to and including 50 µg per plate did not cause any bacteriotoxic effects. Total bacteria counts remained unchanged and no inhibition of growth was observed. At higher doses, the substance had only a weak, strain-specific bacteriotoxic effect. Due to the weakness of this effect this range could nevertheless be used for assessment purposes.
Evidence of mutagenic activity of AE 0035073 00 1B97 0001 was not seen. No biologically relevant increase in the mutant count, in comparison with the negative controls, was observed.
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxtde and 2-aminoanthracene had a marked mutagenic effect, as was seen by a biologically relevant increase in mutant colonies compared to the corresponding negative controls.

Therefore, AE 0035073 00 1B97 0001 was considered to be non-mutagenic without and with S9 mix in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

I. Materials and Methods

A. Material

1. Test Material:	AE 0035073 00 1B97 0001
Description:	White powder
Lot/Batch:	5E0551
Purity:	94.0%
CAS number	52918-63-5
Stability of test compound:	Stable in the vehicle at room temperature at concentrations ranging from 0.01 mg/mL to 50 mg/mL for at least 4 hours.
2. Control materials:	
Negative:	Culture medium
Solvent:	DMSO
Positive:	Sodium azide (Serva) for TA 1535 at 10 µg/plate, Nitrofurantoin (Sigma) for TA 100 at 0.2 µg/plate, 4-Nitro-1,2-phenylene diamine (Merck, Schuchardt) for TA 1537 at 10 µg/plate and TA 98 at 0.5 µg/plate, Mitomycin C (Fluka) for TA 102 at 0.2 µg/plate only in plate incorporation plate, Cumene hydroperoxide (Sigma) for TA 102 in pre-incubation trials only at 50 µg/plate, 2-Aminoanthracene (Fluka) for the activating effect of the S9 mix in all strains at 3 µg/plate
3. Test organisms:	
Species:	Salmonella typhimurium LT2 mutants
Strain:	Histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102
Source:	Strains obtained [redacted] in 1997 and stored in the laboratory since then
4. Test compound concentrations:	
Plate incorporation assay:	First assay for all strains with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/plate
Pre-incubation assay:	For TA 1535, TA 1537, TA98, TA100 and TA 102 with or without S9 mix: 16, 50, 158, 500, 1581 and 5000 µg/tube

B. Study Design and methods

The experimental phase of the study was performed between February 10 to 23, 2004 at Bayer Healthcare AG ([redacted]).

The Salmonella/microsome test is an *in vitro* screening method which detects point mutations caused by chemical agents. 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

AE 0035073 00 1B97 0001or the control material were dissolved in 0.1 mL of DMSO. DMSO (0.1 mL) containing AE 0035073 00 1B97 0001 or 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 water bath 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 was performed as pre-incubation of the previously described mixture in a water bath at 37 °C for 20 minutes. At the end of the pre-incubation 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. Assessment criteria

A reproducible and dose-related increase in mutant colonies of at least one strain was considered to be positive. For TA 1535, TA 100 and TA 98, this increase should be about twice that of negative controls, whereas for TA 1537, at least a threefold increase should be reached. For TA 102 an increase of about 100 mutants should be reached. Otherwise, the result was considered as negative.

II. Results and discussion

There was no indication of a bacteriotoxic effect of AE 0035073 00 1B97 0001 at doses of up to and including 50 µg 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. Higher doses had only a weak, strain-specific bacteriotoxic effect. Therefore they could nevertheless be used for assessment purposes.

None of the five strains concerned showed in the plate incorporation test a dose-related and biologically relevant increase in mutant counts over those of the negative controls. This applied both to the tests with and without S9-mix and was confirmed by the results of the pre-incubation trials.

The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, 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 5.8.1-04: Mean mutant values per plate in the plate incorporation assay

Test item	Concentration µg/plate	S9 mix	Strains				
			TA 1535	TA 100	TA 1537	TA 98	TA 102
AE 0035073	0	-	21	154	8	14	171
	16	-	21	158	6	13	186
	50	-	19	150	6	14	193
	158	-	22	155	5	18	183
	500	-	18	164	6	14	180
	1581	-	22	155	5	15	184
	5000	-	23	177	5	16	192
Na-azide	10	-	680				
NF	0.2	-		349			
4-NPDA	10	-			78		
	0.5	-				130	
MMC	0.2	-					559
AE 0035073	0	+	14	174	12	26	247
	16	+	14	187	12	24	243
	50	+	12	184	8	20	233
	158	+	13	189	12	26	218
	500	+	8	64	9	20	177
	1581	+	7	96	9	20	154
	5000	+	9	150	7	20	180
2-AA	3	+	150	1089	317	1091	455

Table 5.8.1-05: Mean mutant values per plate in the pre-incubation assay

Test item	Concentration µg/plate	S9 mix	Strains				
			TA 1535	TA 100	TA 1537	TA 98	TA 102
AE 0035073	0	-	18	132	6	15	242
	16	-	17	138	5	17	230
	50	-	14	149	7	13	231
	158	-	13	162	5	13	245
	500	-	16	160	6	17	223
	1581	-	19	166	6	15	234
	5000	-	21	168	5	18	223
Na-azide	10	-	663				
NF	0.2	-		468			
4-NPDA	10	-			126		
	0.5	-				155	
Cumene	50	-					431
18AE 001735073	0	-	13	190	9	29	287
	16	+	13	200	7	29	290
	50	+	13	173	8	23	219
	158	+	13	205	11	18	210
	500	+	4	46	4	20	192
	1581	+	2	76	7	19	183
	5000	+	2	156		24	189
2-AA14	3	+	173	1372	273	938	433

III. Conclusions

No indication of mutagenic effects of AE 0035073 00 1B97 0001could be found at assessable doses of up to 5000 µg/plate in any of the Salmonella typhimurium strains used in the assay.

In vitro genotoxicity – Test for gene mutation in mammalian cells

Report:	KCA 5.8.1/07; [redacted]; 2013; M-461312-01-1
Title:	Gene mutation assay in Chinese hamster V79 cells in vitro (V79/HPRT) - Trans isomer of deltamethrin AE 0035073
Report No.:	1549101
Document No.:	M-461312-01-1
Guideline(s):	This study was conducted according to the procedures indicated by the following internationally accepted guidelines and recommendations: Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 476: In vitro Mammalian Cell Gene Mutation Test, adopted July 21, 1997; Commission Regulation (EC) No. 440/2008 B17, dated May 30, 2008; United States Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.5300, In Vitro Mammalian Cell Gene Mutation Test, EPA 712-C-98-221, August 1998; Japanese Guidelines: Kanpoan No. 287 - Environment Protection Agency Eisei No. 127 - Ministry of Health & Welfare Heisei 09/10/31 Kikyoku No. 2 - Ministry of International Trade & Industry; Ministry of Agriculture, Forestry and Fisheries of Japan. MAFF Notification No. 12 Nousan-8147, 24 November 2000
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive Summary

The purpose of the study was to assess the point mutagenic potential of the trans isomer of deltamethrin (batch SES 10538-2-2, 94.5 % of purity) at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in V79 cells.

For dose selection, a preliminary cytotoxicity test was conducted with and without an Aroclor 1254-induced rat liver metabolic activation system (S9 mix) using concentrations of the trans isomer of deltamethrin ranging from 39.1 to 5000 µg/mL. No cytotoxic effects were observed. The concentration range of the main experiments was limited by the solubility of the test item in aqueous medium. Precipitation was observed at 78.1 µg/mL and above following 4 and 24 hours treatment with and without metabolic activation. The trans isomer of deltamethrin was tested in both experiments from 4.9 to 156.0 µg/mL.

No substantial and reproducible dose dependent increase of the mutation frequency was observed in either of the two main experiments.

Appropriate reference mutagens, used as positive controls, induced a distinct increase in mutant colonies and thus, showed the sensitivity of the test system and the activity of the metabolic activation system.

In conclusion it can be stated that under the experimental conditions reported the test item did not induce gene mutations at the HPRT locus in V79 cells. Therefore, Trans Isomer of Deltamethrin AE 0035073 is considered to be non-mutagenic in this HPRT assay.

I. Materials and Methods

A. Material

1. Test Material:	Trans isomer of deltamethrin AE 0035073
Description:	White solid
Lot/Batch:	SES 10538-2-2

Purity:	94.5%
CAS number	52918-63-5
Stability of test compound:	Stable for the duration of the study, Expiry date: 2014-03-01

2. Control materials:

	Negative:	Culture medium: Eagle's minimal essential medium supplemented with Hank's salts, 5 µg/mL of Neomycin, 1% of Amphotericin B and 10% foetal calf serum (FCS)
	Solvent:	Tetrahydrofuran (THF) for the trans isomer- DMSO for Dimethylbenzanthracene- not exceeding 0.5% (v/v) in the culture medium. No solvent needed for ethyl methanesulfonate as it is a liquid
	Positive:	Ethyl methanesulfonate (EMS), a directly alkylating agent, used at a final concentration of 150 µg/mL in non-activation trials. Dimethylbenzanthracene (DMBA), promutagen requiring a metabolic activation, used at a final concentration of 1.1 µg/mL, for trials with S9 mix.

3. Test organisms:

Cell line:	Chinese hamster V79 lung cells
Source:	Cells supplied [redacted] Germany and stored in liquid nitrogen in the cell bank [redacted] allowing the repeated use of the same cell culture batch in experiments. They have a modal chromosome number of 22 and a rapid population doubling time (12 to 16 hours).
Culture condition:	Incubation performed at 37 °C in a humidified atmosphere with about 1.5% CO_2

4. Test compound concentrations:

The trans isomer was used at 39.1, 78.1, 156.3, 312.5, 625, 1250, 2500 and 5000 µg/mL in the clonal cytotoxicity assay and at 9.8, 19.5, 39, 78 and 156 µg/mL in the mutagenic assays

5. Metabolic activation:

The S9 fraction was isolated from the livers of Phenobarbital/β-Naphtoflavone induced male Wistar rats. The preparation was kept frozen at -80 °C. The protein concentration in the S9 preparation was 31.4 mg/mL in the pre-experiment and in experiments I and II.

B. Study Design and methods

The experimental phase of the study was performed from May 14 to June 25, 2013 [redacted], Germany.

The selection of V79 forward mutations is based on the resistance of induced mutants to the purine analogue 6-thioguanine (6-TG). This resistance is a result of a mutation at the X-chromosome-linked HPRT locus rendering the cells unable to use 6-TG for DNA synthesis. Therefore, cell colonies formed in the presence of 6-TG are considered to represent mutants at the HPRT gene.

1. Determination of cytotoxicity

The general culture conditions and experimental conditions in this pre-test were the same as described for the mutagenicity experiment below. In this pre-test the colony forming ability of approximately 500 single cells (duplicate cultures per concentration level) after treatment with the test item was observed and compared to the controls.

Toxicity of the test item is indicated by a reduction of the cloning efficiency (CE).

2. Treatment protocol

Thawed stock cultures were propagated at 37 °C in 80 cm^2 plastic flasks. About 5 x 10^5 cells were seeded into each flask with 15 mL of MEM (minimal essential medium) containing Hank's salts, neomycin (5 µg/mL) and Amphotericin B (1 %). The cells were sub-cultured twice weekly. The cell cultures were incubated at 37 °C in a 1.5% carbon dioxide atmosphere (98.5% air). For the selection of mutant cells the complete medium was supplemented with 11 µg/mL 6-thioguanine.

Two to three days after sub-cultivation stock cultures were trypsinized at 37 °C for 5 minutes. Then the enzymatic digestion was stopped by adding complete culture medium with 10% FBS and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.2% in PBS. The cell suspension was seeded into plastic culture flasks at approximately 1.5 x 10^6 (single culture) and 5 x 10^2 cells (in duplicate).

After 24 hours the medium was replaced with serum-free medium containing the test item, either without S9 mix or with 50 µL/mL S9 mix. Concurrent solvent and positive controls were treated in parallel. After 4 hours this medium was replaced with complete medium following two washing steps. In the second experiment the cells were exposed to the test item for 24 hours in complete medium, supplemented with 10% FBS, in the absence of metabolic activation.

Three or four days after treatment 1.5 x 10^6 cells per experimental point were sub-cultivated in 175 cm^2 flasks containing 30 mL medium. Following the expression time of 7 days five 80 cm^2 cell culture flasks were seeded with about 3 - 5 x 10^5 cells each in medium containing 6-TG. Two additional 25 cm^2 flasks were seeded with approx. 500 cells each in non-selective medium to determine the viability. The cultures were incubated at 37 °C in a humidified atmosphere with 1.5% CO_2 for about 8 days. The colonies were stained with 10% methylene blue in 0.01% KOH solution. The stained colonies with more than 50 cells were counted. In doubt the colony size was checked with a preparation microscope.

3. Acceptance criteria

The gene mutation assay was considered acceptable if it met the following criteria:

- The numbers of mutant colonies per 10^6 cells found in the solvent controls fell within the laboratory historical control data range
- The positive control substances must produce a significant increase in mutant colony frequencies
- The cloning efficiency II (absolute value) of the solvent controls must exceed 50%.

The data of this study complied with the above mentioned.

4. Assessment criteria

A test item was classified as positive if it induced either a concentration-related increase of the mutant frequency or a reproducible and positive response at one of the test points.

A test item producing neither a concentration- related increase of the mutant frequency nor a reproducible positive response at any of the test points was considered to be non-mutagenic in this system.

A positive response was described as follows:

A test item was classified as mutagenic if it reproducibly induced a mutation frequency that was three times above the spontaneous mutation frequency at least at one of the concentrations in the experiment.

The test item was classified as mutagenic if there was a reproducible concentration-related increase of the mutation frequency. Such evaluation may be considered also in the case that a threefold increase of the mutant frequency was not observed.

However, in a case by case evaluation this decision depends on the level of the corresponding solvent control data. If there was by chance a low spontaneous mutation rate within the laboratory's historical control data range, a concentration-related increase of the mutations within this range has to be discussed. The variability of the mutation rates of solvent controls within all experiments of this study was also taken into consideration.

5. Statistical analysis

A linear regression (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. The number of mutant colonies obtained for the groups treated with the test item were compared to the solvent control groups. A trend is judged as significant whenever the p-value (probability value) is below 0.05.

II. Results and discussion

The test item Trans Isomer of Deltamethrin AE 0035073 was assessed for its potential to induce gene mutations at the HPRT locus using V79 cells of the Chinese hamster.

The assay was performed in two independent experiments, using two parallel cultures each. The first main experiment was performed with and without liver microsomal activation and a treatment period of 4 hours. The second experiment was performed with a treatment time of 4 hours with and 24 hours without metabolic activation.

The cell cultures were evaluated at the following concentrations: 9.8, 19.5, 39, 78 and 156 µg/mL.

Precipitation of the test item at the end of treatment was noted in both main experiments at 78.0 µg/mL and above with and without metabolic activation.

No relevant cytotoxic effect indicated by a relative cloning efficiency I and/or relative cell density below 50% in both parallel cultures occurred up to the maximum concentration with and without metabolic activation following 4 and 24 hours treatment.

No relevant and reproducible increase in mutant colony numbers/10^6 cells was observed in the main experiments up to the maximum concentration. The mutation frequency remained well within the historical range of solvent controls.

A linear regression analysis (least squares) was performed to assess a possible dose dependent increase of mutant frequencies. No significant dose dependent trend of the mutation frequency indicated by a probability value of <0.05 was determined in any of the experimental groups.

In both experiments of this study (with and without S9 mix) the range of the solvent controls was from 8.5 up to 34.8 mutants per 10^6 cells; the range of the groups treated with the test item was from 2.8 up to 34.4 mutants per 10^6 cells.

EMS (150 µg/mL) and DMBA (1.1 µg/mL) were used as positive controls and showed a distinct increase in induced mutant colonies.

Table 5.8.1.-06: HPRT assaywith or without metabolic activation - experiment 1

Concentration µg/mL	Relative cloning efficiency I (survival) %	Relative cloning efficiency II (viability)%	Mutant colonies/10^6 cells	Induction factor
Experiment I/4 hour treatment without S9				
Solvent control with THF	100 100	100 100	34.8 12.7	1.0 1.0
Positive control (EMS 150)	112.8 94.0	113.1 109.2	127.8 116.5	3.7 9.2
Trans isomer 4.9	87.2 73.9	Culture not continued		
9.8	88.8 65.7	93.9 109.8	23.7 11.7	0.7 0.9
19.5	87.8 24.8	87.0 110.8	10.4 11.8	0.3 0.9
39.0	55.9 76.1	88.7 123.8	30.7 7.7	0.9 0.6
78.0	87.3 67.6	89.4 100.0	19.5 20.3	0.6 1.6
156.0	94.7 98.0	91.9 104.2	25.1 19.4	0.7 1.5
Experiment I/4 hour treatment with S9				
Solvent control with THF	100.0 100.0	100.0 100.0	17.1 9.0	1.0 1.0
Positive control (DMBA 1.1)	34.0 28.3	51.2 78.0	972.0 782.1	56.9 86.7
Trans isomer 4.9	104.6 106.7	Culture not continued		
9.8	103.9 98.0	72.8 87.5	12.3 23.2	0.7 2.6
19.5	99.1 103.0	67.4 87.3	9.5 21.5	0.6 2.4
39.0	88.7 99.8	92.1 95.5	16.5 12.7	1.0 1.4
78.0	87.1 98.6	99.0 111.7	6.7 18.4	0.4 2.0
156.0	90.5 98.8	60.5 97.1	34.4 13.9	2.0 1.5

Table 5.8.1.-07: HPRT assay with or without metabolic activation - experiment 2

Concentration µg/mL	Relative cloning efficiency I (survival) %	Relative cloning efficiency II (viability)%	Mutant colonies/10^6 cells	Induction factor
Experiment II/24 hour treatment without S9				
Solvent control with THF	100.0 100.0	100.0 100.0	8.5 18.3	1.0 1.0
Positive control (EMS 150)	94.0 97.4	82.9 100.5	345.9 266.9	40.8 14.6
Trans isomer 4.9	102.5 99.0	Culture not continued		
9.8	98.0 100.5	86.3 97.5	15.2 10.7	1.8 0.6
19.5	94.5 94.0	106.5 98.4	9.2 11.3	1.1 0.6
39.0	94.5 96.6	100.3 93.9	10.1 16.2	1.2 0.9
78.0	92.8 92.2	91.5 96.7	14.7 11.1	1.7 0.6
156.0	93.0 91.6	94.8 99.3	10.0 12.4	1.2 0.7
Experiment II/4 hour treatment with S9				
Solvent control with THF	100.0 100.0	100.0 100.0	17.7 10.8	1.0 1.0
Positive control (DMBA 1.1)	83.9 86.9	54.8 116.4	648.7 301.4	36.7 27.8
Trans isomer 4.9	101.8 106.4	Culture not continued		
9.8	102.3 105.2	84.6 113.5	7.2 15.1	0.4 1.4
19.5	103.7 98.3	79.9 110.9	7.6 3.6	0.4 0.3
39.0	98.4 94.3	89.2 100.1	18.1 9.0	1.0 0.8
78.0	99.9 101.8	74.9 122.3	12.3 5.8	0.7 0.5
156.0	103.8 86.6	84.4 128.2	21.8 2.8	1.2 0.3

III. Conclusions

In conclusion it can be stated that under the experimental conditions reported the trans isomer of deltamethrin did not induce gene mutations at the HPRT locus in V79 cells.

In vitro genotoxicity - Test for clastogenicity in mammalian cells

Report:	KCA 5.8.1/08; [redacted]; 2013; M-469011-01-1
Title:	Trans isomer of deltamethrin AE 0035073: In vitro chromosome aberration test in Chinese hamster V79 cells
Report No.:	1549102
Document No.:	M-469011-01-1
Guideline(s):	OECD No. 473 (adopted July 21, 1997) EC No. 440/2008, B10 dated May 30, 2008 EPA OPPTS 870.5375, EPA 712-C-98-223, August 1998 MAFF of Japan
Guideline deviation(s):	none
GLP/GEP:	**yes**

Executive Summary

In this *in vitro* assessment of the clastogenic potential of the trans isomer of deltamethrin AE 0035073 (batch SES 10538-2-2, 94.5% of purity), Chinese Hamster V79 cells were exposed to AE 0035073 at 19.5, 39.1, 78.1, 156.3, 312.5, 625.0, 1250.0, 2500.0 and 5000 µg/mL, dissolved in tetrahydrofuran, (THF, 0.5% in culture medium). For each dose level, duplicate cultures were used in both the presence and absence of a metabolic activation system (S9 mix). THF was also used as a negative control. Ethylmethane sulfonate, which produces crosslinks in the DNA, and cyclophosphamide, which induces chromosomal damage after metabolic activation, were used as positive controls. After 4 hours treatment, the medium was changed and the cells were harvested 14 hours later. An additional experiment was performed using continuous treatment for 18 hours, harvest at the same time, at AE 0035073-concentrations of 19.5, 39.1, 78.1, 156.3, 312.5, 625.0, 1250.0, 2500.0 and 5000 µg/mL. Colcemid was added to each flask two to three hours prior to harvest to arrest the cells in a metaphase-like stage of mitosis.

In both cytogenetic experiments, in the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.
In both independent experiments, no biologically relevant increase in the number of cells carrying structural chromosomal aberrations was observed after treatment with the test item. However, one single statistically significant increase was observed in Experiment I in the absence of S9 mix after treatment with 156.3 Kg/mL (3.0 % aberrant cells, excluding gaps). Since this value was clearly within the range of the laboratory historical control data (0.0 – 4.0 % aberrant cells, excluding gaps) the finding has to be regarded as biologically irrelevant.
No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.
Appropriate mutagens were used as positive controls. They induced statistically significant increases in cells with structural chromosome aberrations.
In conclusion, it can be stated that under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in V79 cells *in vitro*.
Therefore, Trans Isomer of Deltamethrin AE 0035073 is considered to be non-clastogenic in this chromosome aberration test, when tested up to precipitating concentrations.

I. Materials and Methods

A. Material

1. Test Material:	Trans isomer of deltamethrin, AE 0035073
Description:	White solid
Lot/Batch:	SES 10538-2-2
Purity:	94.5%
CAS	52918-63-5
Stability of test compound:	No analysis performed during the study

2. Control materials:	Negative:	Culture medium
	Solvent:	THF for AE 0035073
	Positive:	Ethylmethane sulfonate without S9 mix at 1000 µg/mL for experiment I,and 600 µg/mL for experiment II Cyclophosphamide with S9 mix at 1.4 µg/mL for experiment I and 1.0 µg/mL for experiment II

3. Test organisms:

Cell line:	Chinese hamster V79 lung cells
Source:	Cells supplied by Laboratory [redacted] Germany
Culture condition:	Incubation performed at 37 °C in a humidified atmosphere with about 1.5% CO_2

4. Test compound concentrations:

<u>In experiments I and II:</u> AE 0035073was used at 19.5, 39.1, 78.1, 156.3, 312.5, 625.0, 1250.0, 2500.0 and 5000 µg/mL.

B. Study design and methods

The experimental phase of the study was performed from May 15 to June 25, 2013 [redacted] Germany.

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

A preliminary cytotoxicity test was performed to determine the concentrations to be used in the main experiment. Cytotoxicity is characterized by the percentages of mitotic suppression and/or reduction in cell number in comparison to the controls by counting 1000 cells per culture in duplicate. The experimental conditions in this pre-test phase were identical to those required and described below for the main experiment.

The pre-test was performed with 9 concentrations of the test item separated by no more than a factor of V10 and a solvent and positive control. All cell cultures were set up in duplicate. Exposure time was 4 hrs (with and without S9 mix). The preparation interval was 18 hrs after start of the exposure.

Test item concentrations between 19.5 and 5000.0 µg/mL (with and without S9 mix) were chosen for the evaluation of cytotoxicity. In the pre-test for toxicity, precipitation of the test item was observed at the end of treatment at 312.5 µg/mL and above. Since the cultures fulfilled the requirements for cytogenetic evaluation, this preliminary test was designated Experiment I.

Using reduced mitotic indices/cell numbers as an indicator for toxicity in Experiment I, no cytotoxic effects were observed after 4 hours treatment in the absence and presence of S9 mix. Therefore, 5000.0 µg/mL was chosen as top treatment concentration for Experiment II.

2. Seeding of the cultures

Thawed stock cultures were propagated at 37 °C in 80 cm2 plastic flasks. About 5 x 10^5 cells per flask were seeded in 15 mL of MEM (minimal essential medium) containing Hank's salts, glutamine and Hepes (25 mM). Additionally, the medium was supplemented with penicillin/streptomycin (100 U/mL/100 Kg/mL) and 10 % (v/v) fetal bovine serum (FBS). The cells were sub-cultured twice a week.

Exponentially growing stock cultures more than 50 % confluent were rinsed with Ca-Mg-free salt solution containing 8000 mg/L NaCl, 200 mg/L KCl, 200 mg/L KH_2PO_4 and 150 mg/L Na_2HPO_4. Afterwards the cells were treated with trypsin-EDTA-solution at 37 °C for approx. 5 minutes. Then, by adding complete culture medium including 10 % (v/v) FBS the enzymatic treatment was stopped and a single cell suspension was prepared. The trypsin concentration for all sub-culturing steps was 0.25 % (w/v) in Ca-Mg-free salt solution. For experimental performance the cells were seeded into Quadriperm dishes containing microscopic slides. Into each chamber 1 x 10^4 – 6 x 10^4 cells were seeded with regard to the preparation time.

All incubations were done at 37 °C in a humidified atmosphere with 1.5 % carbon dioxide (98.5 % air).

3. Treatment protocol

Exposure period 4 hours

The culture medium of exponentially growing cell cultures was replaced with serum-free medium containing the test item. For the treatment with metabolic activation 50 µL S9 mix per mL culture medium were added. Concurrent solvent and positive controls were performed. After 4 hours the cultures were washed twice with saline. The cells were then cultured in complete medium containing 10 % (v/v) FBS for the remaining culture time of 14 hours.

Exposure period 18 hours

The culture medium of exponentially growing cell cultures was replaced with complete medium containing 10 % (v/v) FBS including the test item without S9 mix. The medium was not changed until preparation of the cells. Concurrent solvent and positive controls were performed.

4. Preparation of the cultures

Colcemid was added to the culture medium (0.2 µg/mL) approximately two to three hours before the requested harvest time. The cells were treated, 2.5 hours later, on the slides in the chambers with hypotonic solution (0.4% KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with a mixture of methanol and glacial acetic acid (3+1 parts, respectively). After

preparation the cells were stained with Giemsa and labelled with a computer-generated random code to prevent scorer bias.

5. Evaluation of cell numbers

The evaluation of cytotoxicity indicated by reduced cell numbers was made after the preparation of the cultures on spread slides. The cell numbers were determined microscopically by counting 10 defined fields per coded slide. The cell number of the treatment groups is given in percentage compared to the respective solvent control.

6. Analysis of metaphase cells

At least 100 well-spread metaphases were evaluated per culture for structural aberrations, except for the positive controls in Experiment I in the presence of S9 mix and Experiment II in the absence of S9 mix, where only 50 metaphases were evaluated. Only metaphases containing a number of centromeres equal to a number of 22 ± 1 were included in the analysis. Breaks, fragments, deletions, exchanges and chromosomal disintegrations are recorded as structural chromosomal aberrations. Gaps were recorded as well, but they are not included in the calculation of the aberration rates since gaps are achromatic lesions of unknown biological relevance for which a clear relationship to treatment cannot be established.

7. Evaluation criteria

A test item was classified as non-clastogenic if:

- The number of induced structural chromosome aberrations in all evaluated dose groups was in the range of the laboratory's historical control data,

and/or

- no significant increase of the number of structural chromosome aberrations was observed.

A test item is classified as clastogenic if:

- The number of induced structural chromosome aberrations is not in the range of the laboratory's historical control data,

and

- either a concentration-related or a significant increase of the number of structural chromosome aberrations is observed.

Statistical significance was confirmed by means of the Fisher's exact test ($p < 0.05$). However, both biological and statistical significance should be considered together. If the criteria mentioned above for the test item were not clearly met, the classification with regard to the historical data and the biological relevance was discussed and/or a confirmatory experiment was performed.

8. Assessment criteria

The chromosome aberration test was considered acceptable if it met the following criteria:

- The number of structural aberrations found in the solvent controls fell within the range of the laboratory's historical control data.
- The positive control substances produced significant increases in the number of cells with structural chromosome aberrations, which were within the range of the laboratory's historical control data.

II. Results and discussion

The test item Trans Isomer of Deltamethrin AE 0035073, dissolved in THF, was assessed for its potential to induce chromosomal aberrations in V79 cells *in vitro* in the absence and presence of metabolic activation by S9 mix.

Two independent experiments were performed. In Experiment I the exposure period was 4 hours with and without S9 mix. In Experiment II the exposure period was 4 hours with S9 mix and 18 hours without S9 mix. The chromosomes were prepared 18 hours (Exp. I & II) after the start of treatment with the test item.

In each experimental group two parallel cultures were analysed. At least 100 metaphases per culture were scored for structural chromosomal aberrations, except for the positive controls in Experiment I in the presence of S9 mix and Experiment II in the absence of S9 mix, where only 50 metaphases were evaluated. 1000 cells were counted per culture for determination of cytotoxicity.

The highest treatment concentration in this study, 5000.0 µg/mL was chosen with regard to the solubility properties of the test item and with respect to the OECD Guideline for *in vitro* mammalian cytogenetic tests.

Visible precipitation of the test item in the culture medium was observed at 312.5 Kg/mL and above in all experimental parts in the absence and presence of S9 mix at the end of treatment. No relevant influence on osmolarity or pH value was observed.

In both cytogenetic experiments, in the absence and presence of S9 mix, no cytotoxicity was observed up to the highest applied concentration.

In both experiments, in the absence and presence of S9 mix, no biologically relevant increase in the number of cells carrying structural chromosome aberrations was observed. The aberration rates of the cells after treatment with the test item (0.5 – 3.5 % aberrant cells, excluding gaps) were close to the range of the solvent control values (0.5 – 2.0 % aberrant cells, excluding gaps) and within the range of the laboratory historical solvent control data. However, one single statistically significant increase was observed in Experiment I in the absence of S9 mix after treatment with 156.3 µg/mL (3.0 % aberrant cells, excluding gaps). Since this value was clearly within the range of the laboratory historical control data (0.0 – 4.0 % aberrant cells, excluding gaps) the finding has to be regarded as biologically irrelevant.

No evidence of an increase in polyploid metaphases was noticed after treatment with the test item as compared to the control cultures.

In both experiments, either EMS (1000 or 600 µg/mL) or CPA (1.0 or 1.4 µg/mL) were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

Table 5.8.1.-08: Summary of the results of the chromosomal aberration study with AE 0035073

Experiment	Test item concentration	Cell number in % of control	Mitotic indices in % of control	Aberrant cells in %		
				Including gaps [£]	Excluding gaps [£]	With exchanges
Exposure period of 4 hours without S9 mix						
I	THF 0.5%	100.0	100.0	2.0	0.5	0.0
	EMS 1000 µg/mL	n.d.	103.7	14.0	**13.0 ***	8.0
	78.1 µg/mL	89.1	86.6	3.0	2.0	0.5
	156.3 µg/mL	92.1	93.7	4.0	**3.0 ***	0.5
	312.5 µg/mL[P]	94.7	111.5	2.5	2.5	0.0
Exposure period of 18 hours without S9 mix						
II	THF 0.5%	100.0	100.0	2.0	2.0	0.0
	EMS 600 µg /mL[$]	n.d.	69.6	50.0	**50.0 ***	15.0
	78.1 µg/mL	89.8	138.7	2.0	2.0	0.5
	156.3 µg/mL	95.4	112.6	1.0	1.0	0.5
	312.5 µg/mL[P]	86.7	137.0	3.0	3.0	1.5
Exposure period of 4 hours with S9 mix						
I	THF 0.5%	100.0	100.0	1.5	1.5	0.0
	CPA 1.4 µg /mL[$]	n.d.	76.5	41.0	**39.0 ***	13.0
	78.1 µg/mL	99.1	124.3	0.5	0.5	0.0
	156.3 µg/mL[$$]	95.3	96.6	4.3	3.3	0.5
	312.5 µg/mL[P]	98.2	103.4	3.0	2.5	1.0
II	THF 0.5%	100.0	100.0	2.0	2.0	0.5
	CPA 1.0 µg /mL	n.d.	75.6	18.0	**17.0 ***	7.5
	78.1 µg/mL	93.0	106.0	1.5	0.5	0.5
	156.3 µg/mL	88.3	98.7	4.0	3.5	0.5
	312.5 µg/mL[P$$]	94.1	115.7	4.0	3.3	0.0

[£]: inclusive cells carrying exchanges [$]: Evaluation of 50 metaphases per culture [$$]: evaluation of 200 metaphases per culture n.d. :not determined * : aberration frequency statistically higher than corresponding control values

III. Conclusions

In conclusion, it can be stated that under the experimental conditions reported, the test item Trans isomer of deltamethrin AE 0035073 did not induce structural chromosome aberrations in *V79* cells (Chinese hamster cell line) when tested up to precipitating concentrations.

CA 5.8.2 Supplementary studies on the active substance

To comply with new US-EPA requirement, a 28-day immunotoxicity study was performed in the female Sprague-Dawley rats. Deltamethrin was administered continuously via the diet to separate

groups of 10 females per group at concentrations of 100, 300 and 600 ppm (equating approximately to 8.3, 23.5, 48.3 mg/kg body weight/day) for at least 28 days. A similarly constituted group received untreated diet and acted as a control group. An additional group of 10 female rats was administered cyclophosphamide (immunosuppressive agent) daily by gavage for at least 28 days at a dose of 3.5 mg/kg body weight/day and acted as positive control group. On Study Day 26, four days before necropsy, all animals were immunized with Sheep Red Blood Cell (SRBC) antigen by intravenous injection. On Study Day 30 (just before necropsy), blood samples were collected from the retro-orbital venous plexus of each animal for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighed. No impairment of the immunological IgM response following immunization with SRBC was observed in animals treated with deltamethrin at dose levels up to 600 ppm for at least 28 days. Therefore, deltamethrin was considered not to have any immunotoxic potential.

The pharmacokinetic behavior of deltamethrin and the potential influence of the matrix on the test compound blood levels were investigated in a study performed by [redacted] in 2009 (M-356672-01-2). Deltamethrin administered in an aqueous rodent diet suspension (slurry) to rats was rapidly absorbed, when compared to the corn oil suspension. For the three dose levels (0.3, 1.0 and 3.0 mg/kg), mean Tmax were shorter in the case of the aqueous slurry compared to those observed for the corn oil vehicle, but maximal concentrations were much lower for the aqueous than for the corn oil preparation. Elimination phases were also shorter in the aqueous slurry when compared to the corn oil suspension.

Table 5.8.2-01: Supplementary studies on deltamethrin

Type of study (Document N°) Dose range	NOEL/NOAEL		LOAEL		Comments
	ppm	mg/kg/d	ppm	mg/kg/d	
28-day immunotoxicity [redacted] 2012 M-428263-01-1 0, 100, 300, 600 ppm	600	48.3	>600	>48.3	No immunotoxic potential
Plasma kinetic study [redacted] 2009 M-356672-01-2			-	-	Rapid absorption, shorter Tmax and rapid elimination in the aqueous suspension compared to the corn oil suspension

Report:	KCA 5.8.2/02; [redacted]; 2012; M-428263-01-1
Title:	Deltamethrin - 28-day immunotoxicity study in the female Sprague-Dawley rat by dietary administration
Report No.:	SA 10360
Document No.:	M-428263-01-1
Guideline(s):	U.S.E.P.A., OPPTS Series 870, Health Effects Testing Guidelines, No 870.7800 (August 1998)
Guideline deviation(s):	not specified
GLP/GEP:	**yes**

Executive Summary

Deltamethrin (batch number: ABKBDCK008, a white solid, 99.8% w/w purity) was administered continuously via dietary administration to separate groups of female Sprague-Dawley rats (10/group) at concentrations of 100, 300 and 600 ppm (equating approximately to 8.3, 23.5, 48.3 mg/kg body weight/day) for at least 28 days. A similarly constituted group received untreated diet and acted as a control group. An additional group of 10 female rats was administered cyclophosphamide (immunosuppressive agent) daily by gavage for at least 28 days at a dose of 3.5 mg/kg body weight/day and acted as positive control group.

Animals were observed daily for mortality and clinical signs. Body weight and food consumption were recorded once weekly. A detailed physical examination was performed once during the acclimatization phase and at least weekly throughout the study. On Study Day 26, four days before necropsy, all animals were immunized with Sheep Red Blood Cell (SRBC) antigen by intravenous injection of 2.5 x 10^9 SRBC/animal via the tail vein. On Study Day 30 (just before necropsy), blood samples were collected from the retro-orbital venous plexus of each animal for specific anti-SRBC immunoglobulin M (IgM) analysis. All animals were necropsied, gross pathology observations were performed and selected organs (spleen and thymus) weighed.

Deltamethrin induced no treatment-related mortality and no treatment-related clinical signs. Specific anti-SRBC IgM levels were unaffected by the test item administration at all dietary levels.

At 600 and 300 ppm of deltamethrin, mean body weight and mean body weight gain appeared to be lower, compared to the control group, although statistical significance ($p \leq 0.05$) on the cumulative body weight gain was only noted after treatment with the mid-dose of 300 ppm (-21% at 300 ppm compared to -13% at 600 ppm).

At 100 ppm, there was no treatment-related effect.

For immunological response, the results obtained in control animals after immunization with the antigen SRBC and those obtained with the positive control item confirmed the ability of the system to detect the immune-suppressive effects and confirmed the validity of the study design.

Up to the highest dose tested of 600 ppm of deltamethrin, no relevant change was noted in anti-SRBC IgM concentrations, compared to controls.

In conclusion, no impairment of the immunological IgM response following immunization with SRBC was observed in animals treated with deltamethrin at dose levels up to 600 ppm for at least 28 days. Therefore, deltamethrin was considered not to have an immunotoxic potential.

I. MATERIALS AND METHODS

A. Materials:

1. Test Material:	Deltamethrin
Description:	white solid
Lot/Batch:	ABKBDCK008
Purity:	99.8%
CAS:	52918-63-5
Stability of test compound:	Stable at 100 and 600 ppm in rodent diet over a frozen storage period of 33 days followed by 10 days storage at room temperature
2. Vehicle and /or positive control:	Cyclophosphamide
Description:	white powder
Lot/Batch:	120M1253
Purity:	100.6%
CAS:	6055-19-2
Stability of test compound:	Stable at 0.1, 1and 3g/L in a previous study for a time period which covers the period of storage and usage for the [redacted] study
3. Test animals:	
Species:	Female Rat
Strain:	Crl:CD(SD)
Age:	7 weeks
Weight at dosing:	161 to 218 g for females
Source:	[redacted], France
Acclimation period:	12 days
Diet:	Powdered and irradiated diet A04CP1-10 from [redacted] France) ad libitum
Water:	Filtered and softened tap water, ad libitum
Housing:	Rats were housed individually in suspended, stainless steel, wire-mesh cages
Environmental conditions:	Temperature: 22 ± 2 °C Humidity55: 55 ± 15% Air changes: Approximately 10 to 15 changes per hour Photoperiod: Alternating 12-hour light and dark cycles (7 am - 7 pm)

B. Study design:

1. In life dates

September 14, 2011 to October 25, 2011 performed at [redacted] France.

2. Animal assignment and treatment

Fifty-five female Sprague-Dawley [redacted] rats were obtained from [redacted], France.

They were acclimatized to laboratory conditions for twelve days prior to the treatment and were approximately 7 weeks old at the start of treatment. All animals were weighed at least weekly and checked daily for clinical signs, moribundity and mortality. At the time of randomization, all animals were weighed. An automatic procedure (XMS Path-Tox Version 4.2.2) was used to select animals for the study from the middle of the weight range of the available animals, ensuring a similar body weight distribution among groups. Fifty female rats were selected for the study. Selected animals were in a weight range from 161 to 218 g for the females on the start of treatment.

The dose levels of 0, 100, 300 and 600 ppm were set after evaluation of the general systemic toxicities seen in previous studies conducted with this substance.

Table 5.8.2-02: Study design

Group	Test Substance	Dose level (ppm)	Number of animals Per group
		Female	
1	Control	0	10
2	Deltamethrin	100	10
3		300	10
4		600	10
Group	Positive control	Dose level (mg/kg/day)	Number of animals per group
5	Cyclophosphamide	3.5	10

All groups treated by the test substance received the appropriate dietary concentrations at a constant dose level. Control group and the group treated by the immunosuppressive agent cyclophosphamide received untreated diet.

Rats received the cyclophosphamide formulation by gavage (3.5 mg/kg bw/day) at a dosage volume of 5 mL/kg body weight. The volume administered to each rat was adjusted on the most recently recorded body weight.

3. Diet preparation and analysis of the test substance

The test substance was incorporated into the diet to provide the required dietary concentrations. The test item was ground to a fine powder before being incorporated into the diet by dry mixing. There was one preparation for each concentration. When not in use, the diet formulations were stored at approximately -18° C.

The stability of the frozen dietary formulation was determined during the study at 100 and 600 ppm. The mean value obtained from the homogeneity check was taken as measured concentration. Diet samples from the highest and lowest concentrations were taken and frozen. They were analyzed after having been frozen for 33 days then thawed and kept at room temperature for10 days.
The homogeneity of test substance in diet was verified during the study for the lowest and highest concentrations to demonstrate adequate formulation procedures. The mean value obtained from the homogeneity check was taken as measured concentration. Dietary levels of the test substance were verified for each concentration.
The homogeneity and concentration results ranged between 91 and 98% of the nominal concentration and were therefore within in-house target of 85 and 115% for a dietary mix.
In addition, Deltamethrin was determined to be stable at 100 and 600 ppm in rodent diet over a frozen storage period of 33 days followed by 10 days storage at room temperature.

4. Diet preparation and analysis of the positive control substance (cyclophosphamide)
The dosing formulation of cyclophosphamide was prepared by dissolving the substance in sterilized water to produce the required dosing concentration and stored in air-tight light resistant containers at approximately +5 (± 3 °C) when not in use. There were two preparations during the study.
The homogeneity of cyclophosphamide in vehicle was verified on the first formulation to demonstrate adequate formulation procedures. The mean value obtained from the homogeneity check was taken as measured concentration. Concentration of the positive control substance in vehicle was verified for each preparation.
The stability of cyclophosphamide in vehicle has been demonstrated in previous studies at concentrations of 0.1, 1 and 3 g/l for a time period which covers the period of storage and usage for the current study.

4. Statistics
The following variables were analyzed: body weight parameters, body weight change parameters calculated according to time intervals, average food consumption/day parameters calculated according to time intervals, terminal body weight, absolute and relative organ weights parameters, immunological parameter. Mean and standard deviation were calculated for each group.

Data for the test substance except immunological parameters were analyzed by the Bartlett's test for homogeneity of variances. When the data were homogeneous, an ANOVA was performed followed by Dunnett's test on parameters showing a significant effect by ANOVA. When the data were not homogeneous even after transformation, a Kruskal-Wallis ANOVA was performed followed by the Dunn's test if the Kruskal-Wallis was significant. When one or more group variance(s) equaled 0, means were compared using non-parametric procedures. Group means were compared at the 5% and 1% levels of significance. Statistical analyses were carried out using Path/Tox System V4.2.2. (Module Enhanced Statistics). Immunological parameters were analyzed by a Kruskal Wallis test. If no significance was found the analysis was stopped, if significance was obtained a two-sided Dunn test was performed.
Data for the positive reference substance (cyclophosphamide) except immunological parameters were analyzed by an F test for the homogeneity of variances. When the data were homogeneous, a two-sided T test was performed followed by two-sided modified T test on parameters showing a significant

effect by the F test. When the data were not homogeneous even after transformation, a two-sided modified T test was performed on transformed data. Immunological parameters were analyzed by Mann-Whitney two-sided test.

C. Methods

1. Daily observations

All animals were checked for moribundity and mortality twice daily (once daily on weekends or public holidays). Observed clinical signs were observed at least once daily for the animals exposed to the test substance. Observed clinical signs were recorded at least once daily for animals exposed to the immunosuppressive agent cyclophosphamide. Detailed physical examinations were performed at least weekly during the treatment period. Cages and cage-trays were inspected daily for evidence of ill-health such as blood or loose feces.

2. Body weight

Each animal was weighed at least weekly during the acclimatization period, on the start of treatment (study Day 1), then at weekly intervals throughout the treatment period and before necropsy (terminal body weight).

3. Food consumption

The weight of food supplied and of that remaining at the end of the food consumption period was recorded weekly for all animals during the treatment period.

The weekly mean achieved dosage intake in mg/kg body weight/day for each week and for Weeks 1 to 4 was calculated (except for the group exposed to the immunosuppressive agent cyclophosphamide).

4. Sheep red blood cell (SRBC) sensitization

SRBC characteristics

Identification

Antigen		Sheep Red Blood Cell (SRBC)
Supplier	:	[redacted]
Reference number	:	72 141

Storage

The SRBC was stored at approximately 5 ± 3 °C.

Activity

SRBC was selected as an appropriate antigen, since it has a large size ensuring proper immunization of animals and since it is recommended by the guideline.

d/Preparation

On the day of injection, Sheep Red Blood Cells were washed in PBS (Phosphate Buffered Saline),

counted using a cell counting instrument (Siemens Advia 120) and diluted in PBS in order to obtain a 5 x 10^9 cells/mL preparation. SRBC preparation was kept on ice until use.

SRBC administration

On Study Day 26 after the start of treatment, all animals in all groups were immunized by intravenous injection in the tail vein (0.5 mL/animal) with Sheep Red Blood Cell (SRBC) preparation. Prior to intravenous injection, animals were anesthetized with Isoflurane [redacted] France).

5. Clinical pathology

Blood sampling

Blood samples were taken from all surviving animals in all groups by puncture of the retro-orbital venous plexus 4 days after SRBC immunization (terminal sacrifice). Animals were not diet fasted. Animals were anesthetized by inhalation of Isoflurane [redacted] France). Blood was placed into tubes with clot activator (for serum preparation). After centrifugation, serum aliquots were frozen (approximately -74 °C) until analysis.

SRBC-specific IgM assay

Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the level of SRBC-specific immunoglobulin M in response to antigen administration.

Rat Anti-Sheep Red Blood Cell IgM ELISA kits from Life Diagnostics ([redacted] - USA) were used.

Results were obtained using KC4 (version 3.4 Revision 12).

6. Post-mortem examinations

Necropsy

On Study Day 30, all animals from all groups were sacrificed by exsanguination while under deep anesthesia (Isoflurane inhalation).

All animals were necropsied. The necropsy included the examination of all major organs, tissues and body cavities. Macroscopic abnormalities were recorded but not sampled.

Organ weights

At final sacrifice the following organs were weighed:

- Spleen
- Thymus

II. RESULTS

A. Mortality

No mortality was observed during the study.

B. Clinical Signs

No treatment-related clinical signs were observed during the study at any dietary level.

C. Body weight

At 600 and 300 ppm of deltamethrin, mean body weight and mean body weight gain were reduced compared to the control group, although statistical significance ($p \leq 0.05$) on the cumulative body weight gain was only noted at the mid-dose of 300 ppm (-21% at 300 ppm, compared to -13% at 600 ppm which was not statistically significant).
At 100 ppm, body weight and body weight gain were unaffected by treatment with the test item deltamethrin.

D. Food Consumption

Mean food consumption was unaffected by treatment with the test item deltamethrin at all doses tested.
The mean achieved dose level of deltamethrin expressed as mg/kg body weight/day received by animals during the study were as follows:

Table 5.8.2-03: Mean achieved dietary intake of Deltamethrin (Weeks 1 - 4)

Diet concentration (ppm)	Female (mg/kg/day)
100	8.3
300	23.5
600	48.3

E. SRBC-Specific IgM response

A high inter-individual variability was noted in all the groups, as usually observed with SRBC sensitization. The high mean anti-SRBC IgM concentration observed in the control group confirmed the sensitization of the animals.
No treatment-related change was noted up to and including the highest dietary level of 600 ppm. The slight differences in group mean values observed in the treated groups, relative to the controls, were considered not to be relevant as they were not statistically significant and due to only few values. In addition, there was no consistency across the dose levels and the difference in group mean value from control at 600 ppm was minimal (+18%, compared to +12% at 300 ppm).

Table 5.8.2-04: Mean SRBC-specific IgM

SRBC-specific IgM (µ/mL) mean ± standard deviation (% change when compared to controls)				
Deltamethrin dose level (ppm)	0	125	600	3000
Study Day 30	7816±7799	11602±10689 (+48%)	8771±8993 (+12%)	6430±4440 (-18%)

F. Post-mortem examinations

1. Terminal body weight and organ weight

There was no relevant change in mean terminal body weight in treated females, when compared to the controls.
All organ weight changes were considered to be incidental and not treatment-related.

2. Gross pathology
All the macroscopic changes were considered as incidental and not treatment-related.

G. Positive control

Homogeneity and concentration values of the cyclophosphamide were within the in-house target range (ranging from 96 to 101% of the nominal concentration).
The toxicological results obtained with the use of the positive control cyclophosphamide at the dose level of 3.5 mg/kg/day were in line with those obtained in a previous study in the test facility and in GLP validation studies.
At 3.5 mg/kg/day, when compared to the controls, mean anti-SRBC IgM concentration was markedly lower (-81%, p≤0.01). The magnitude of this difference from controls corresponds to the difference usually observed with cyclophosphamide within our laboratory conditions.
A lower mean terminal body weight was observed in treated females, when compared to the controls (-5%, not statistically significant).
Mean absolute and relative spleen weights were statistically significantly lower, when compared to the controls.

Table 5.8.2-05: Mean spleen weights

Mean spleen weight ±SD at scheduled sacrifice (% change when compared to controls)		
Sex	**Female**	
Dose level of Cyclophosphamide (mg/kg/day)	0	3.5
Mean absolute spleen weight (g)	0.563 ± 0.091	0.414** ± 0.058 (-26%)
Mean spleen to body weight ratio (%)	0.2180 ± 0.0287	0.1693** ± 0.0175 (-22%)

** : p ≤0.01

Atrophic/small thymus and/or spleen were noted in the cyclophosphamide group (5/10 and 8/10, respectively). These changes compare well with those observed in other studies at our laboratory with the administration of cyclophosphamide.

III. CONCLUSION

In conclusion, Deltamethrin was considered not to have any immunotoxic potential in female Sprague Dawley rats when given in the diet at dose levels up to 600 ppm (corresponding to approximately 48.3 mg/kg/day) for at least 28 days.

Report:	KCA 5.8.2/03; [redacted]; 2009; M-356672-01-2
Title:	Deltamethrin: Plasma kinetic study in the male rat by gavage
Report No.:	SA 08235
Document No.:	M-356672-01-2
Guideline(s):	US EPA OPPTS 870.7485
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Executive summary

The aim of this study was to investigate the pharmacokinetic behavior of deltamethrin and the potential influence of the matrix on the test compound blood levels. Deltamethrin, (batch number 27500029, 99.6% w/w purity) was administered orally in a single administration by gavage at three dose levels 0.3, 1.0 and 3.0 mg/kg bodyweight, and using two different vehicles, corn oil or aqueous rodent diet suspension (slurry) to groups of 5 male Wistar rats.
Blood samples were taken at the following time points 0; 0.25; 0.5; 1; 1.5; 2; 4; 6; 8; 24; 30 and 48 hours after administration, by cutting the extreme tip of the tail of the animals. Plasma deltamethrin concentrations were determined using LC/MS/MS equipment.
Following a single oral administration of deltamethrin at the concentrations 0.3, 1.0 and 3.0 mg/kg in corn oil or in aqueous slurry to male Wistar rats, the analysis of the levels in plasma displayed significant differences regarding the absorption and distribution/elimination phases of the test product depending on the vehicle of administration. Deltamethrin administered in an aqueous suspension of rat diet was rapidly absorbed, when compared to the corn oil suspension. For all dose levels, mean Tmax were shorter in the case of the aqueous slurry compared to those observed for the corn oil vehicle, but maximal concentrations were much lower for the aqueous than for the corn oil preparation. Elimination phases were also shorter in the aqueous slurry when compared to the corn oil suspension.

I. MATERIALS AND METHODS

A. Materials:

1. Test Material:	Deltamethrin
Description:	light beige solid
Lot/Batch:	27500029
Purity:	99.6%
CAS:	52918-63-5
Stability of test compound:	Stable at 1 and 21.3 mg/ml in corn oil over 9 days Stable 3 and 1000 ppm in rodent diet
2. Vehicle and /or positive control:	corn oil or rodent diet suspended in drinking water

3. Test animals:

Species:	Male Rat

Strain:	Wistar [redacted]
Age:	9 weeks
Weight at dosing:	290 to 329 g
Source:	[redacted] France
Acclimation period:	12 days
Diet:	Pelleted and irradiated diet A04C-10 [redacted] [redacted] France, ad libitum
Water:	Filtered and softened tap water, ad libitum
Housing:	Rats were housed individually in suspended, stainless steel, wire-mesh cages
Environmental conditions:	Temperature: 22 ± 2 °C Humidity55 : 55 ± 15% Air changes: Approximately 10 to 15 changes per hour Photoperiod: Alternating 12-hour light and dark cycles (7 am - 7 pm)

B. Study design:

1. In life dates:

October 08 to 30, 2008, performed at [redacted] France.

2. Animal assignment and treatment

Thirty four male rats Wistar [redacted] were obtained from [redacted], France. They were acclimatized to laboratory conditions for at least 12 days prior to the treatment and were approximately 9 weeks old at the start of treatment. All animals were examined and weighed at least weekly during the acclimatization phase. At the time of randomization, all animals were weighed. A manual randomization procedure was used to select animals for the study from the middle of the weight range of the available animals that ensured a similar body weight distribution among groups. Body weights were within ±20% of the mean body weight on the day of randomization. Thirty male rats (15 animals for corn oil pact and 15 animals for aqueous suspension) were selected for the study. Selected animals were in a weight range from 290 to 329 g at the start of the exposure to the test substance.

The dose levels of 0, 0.3, 1.0 and 3.0 mg/kg bw were chosen based on published data (Mirfazaelian et al., 2006). Groups of 5 male rats were given the appropriate amount of test substance in the appropriate vehicle as a single oral dose administered by gavage and at a dosage volume of 5 ml/kg body weight.

Table 5.8.2-06: Study design

Group	Test Substance	Dose level (mg/kg bw)	Number of animals Per group (Males)
1	Deltamethrin in corn oil	0.3	5
2		1.0	5
3		3.0	5
5	Deltamethrin in aqueous slurry	0.3	5
6		1.0	5
7		3.0	5

3. Diet preparation and analysis of the test substance

In corn oil: the test substance was incorporated into the corn oil to provide the required concentrations. There were two separate preparations for each concentration as the first formulation (F1) was discarded due to unacceptable analytical results and was replaced by the formulation F1bis. When not in use, the corn oil formulations were stored at room temperature.
In aqueous suspension of rodent diet: the test substance was firstly incorporated into rodent diet which was then suspended in drinking water to reach the concentration levels requested for the study. There was one preparation for each concentration.

The homogeneity of test substance in corn oil and in aqueous suspension was verified before the study for the lowest and highest concentrations to demonstrate adequate formulation procedures. Mean values obtained from the homogeneity checks were used as measured concentrations.
Dietary levels of the test substance were verified for each concentration.
Formulations in corn oil: homogeneity and concentration results ranged between 104 and 112% of the nominal concentrations and were therefore within the in-house target range, except for the homogeneity results obtained at 0.06 g/l (111 or 112%) which were slightly outside of the target range 90-110%. However, these results were considered to be acceptable for the study.
Formulations in aqueous slurry: homogeneity and concentration results ranged between 93 and 105% of the nominal concentrations and were therefore within the in-house target range.
The stability of the test substance in corn oil and in the slurry of feed material were not verified since the slurry formulation was prepared one day prior treatment and previous studies with the active ingredient have verified its stability in powder diet at 3 and 1000 ppm, for a time period which covers the period of storage and usage of the currentt study ([redacted]; 2011; M-270180-03-1). The stability of deltamethrin in corn oil has been verified over a nine day period in a previous study at the dose levels of 1 and 21.3 mg/ml ([redacted]; 2001; M-204103-01-1).

C. Methods

1. Daily observations

All animals were examined at least once daily for mortality and signs of ill health and reaction to treatment throughout the study period. The observations were noted in the appropriate animal room logbook and the study raw data file.

2. Body weight

Each animal was weighed once during the acclimatization period, on the day for randomization and prior to oral administration, in order to calculate the required dosing volume.

3. Blood collection

Before the administration of deltamethrin, a blood sample of approximately 100 to 200 µL was collected from the extreme tip of the tail of each animal, corresponding to the time period "0". Then, blood was collected after dosing at 0.25; 0.5; 1; 1.5; 2; 4; 6; 8; 24; 30 and 48 hours. Blood was placed into tubes containing lithium heparin. Plasma was separated and samples were frozen at about -80°C until processed for deltamethrin quantification using LC-MS/MS.

At the end of the study, plasma from remaining untreated rats were prepared and frozen at about -80°C. These samples were used by the Principal investigator at the Test Site to validate the performance of the analytical method and were also used as quality control samples during the analyses.

4. Plasma analyses for deltamethrin concentration

Plasma samples were kept frozen at approximately -80°C until shipment on dry ice to the analytical Test Site. The analyses were performed at Bayer CropScience, [redacted] under the supervision of the Principal Investigator [redacted]

The samples were then stored at the Test Site at approximately -18 °C. The determination of plasma deltamethrin levels were processed with LC-MS/MS equipment.

5. Post mortem examination

At the end of the blood collection, the animals were euthanized by inhalation of carbon dioxide and discarded without necropsy.

II. RESULTS

A. Mortality

No mortality was observed during the study.

B. Clinical Signs

Liquid and mucoïd faeces were observed for several animals after the administration of Deltamethrin in corn oil. This was considered to be vehicle rather than compound related.

C. Plasma analyses for deltamethrin concentration

Whatever the vehicle, corn oil or aqueous suspension, the concentrations of deltamethrin increased with dose levels but were not proportional to the dose ratio, except in corn oil for 0.3 mg/kg and 1.0 mg/kg.

Deltamethrin concentrations measured in the plasma after a single oral administration of 0.3, 1.0 and 3.0 mg/kg in corn oil and in aqueous slurry displayed significant differences regarding the absorption and distribution/elimination phases of the test product in the male Wistar

rat:

Deltamethrin in the aqueous suspension of rat diet was rapidly absorbed, when compared to the corn oil suspension: for all dose levels, mean Tmax were shorter than those observed in corn oil, but maximal concentrations were much lower for the aqueous than for the corn oil preparation. Elimination phases were also shorter with the aqueous slurry when compared to the corn oil suspension.

Figure 5.8.2-01: Plasma concentrations of deltamethrin administered to rats in corn oil

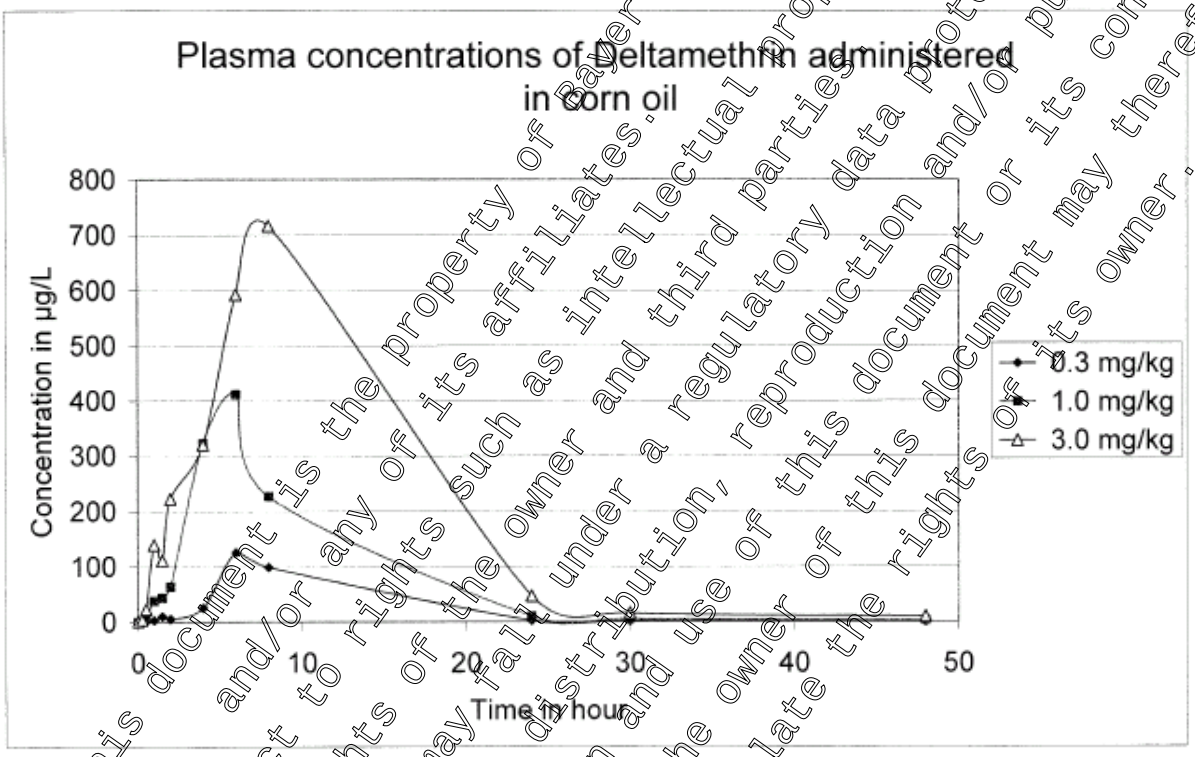
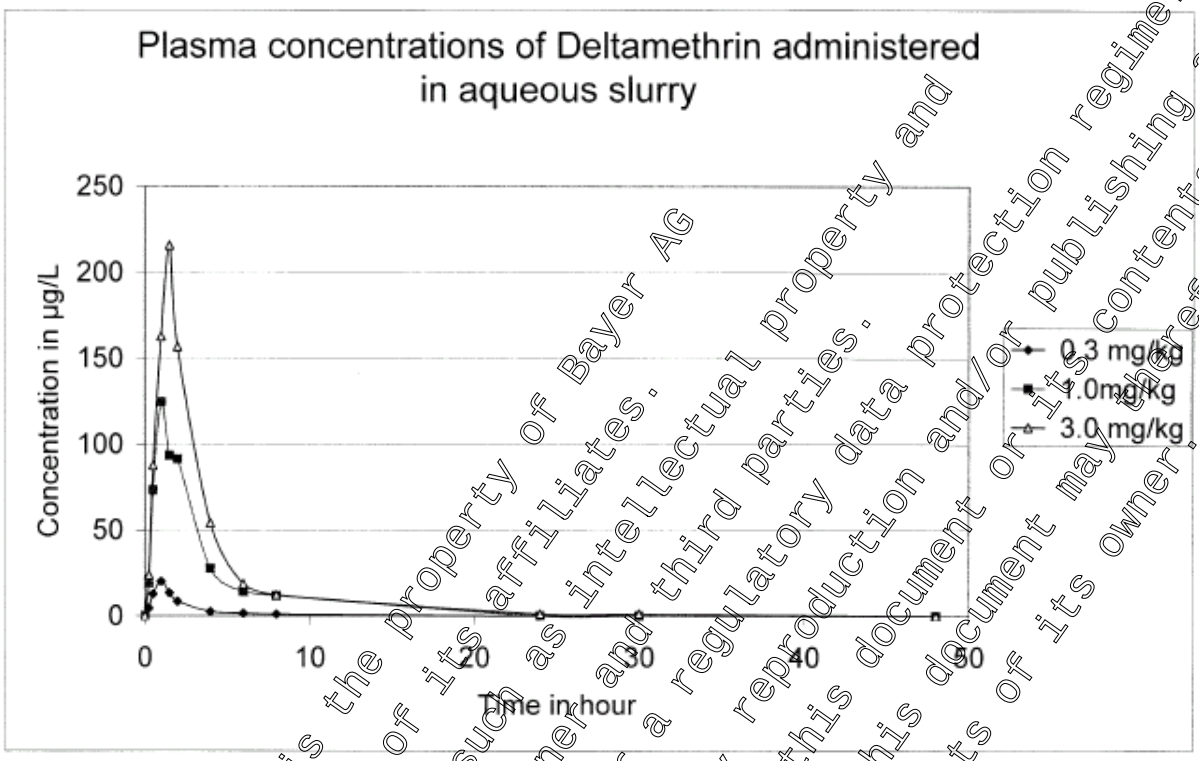


Figure 5.8.2-02: Plasma concentrations of deltamethrin administered to rats in aqueous slurry



III. CONCLUSION

Following a single oral administration of deltamethrin at the concentrations 0.3, 1.0 and 3.0 mg/kg in corn oil or in an aqueous slurry to male Wistar rats, the analysis of the levels in plasma displayed significant differences regarding the absorption and distribution/elimination phases of the test product depending on the vehicle used. Deltamethrin in the aqueous suspension of rat diet was rapidly absorbed, when compared to the corn oil suspension: for all dose levels, mean Tmax were shorter than those observed with corn oil, but maximal concentrations were much lower for the aqueous than for the corn oil preparation.
Elimination phases were also shorter with the aqueous slurry when compared to the corn oil suspension.

CA 5.8.3 Endocrine disrupting properties

Deltamethrin toxicology database has been updated over the past years with a number of OECD and US EPA guideline studies. Deltamethrin has no effects on reproductive indices nor fertility nor reproductive tissues and organs as shown in the multi-generation study in rats. No teratogenic effects were reported in rats or rabbits. No effects on any endocrine organs or reproductive tissues were observed in rats or mice in long term studies ([redacted]; 2006; M-263733-01-1 referenced as KCA 5.8.3.(01).
So, after a detailed analysis of all these apical toxicological studies under inclusion of scientific and regulatory hazard principles in discussion at present, no evidence of any endocrine effect was seen and deltamethrin does not fall under the interim definition for endocrine disruption. Therefore, based on a complete toxicological data set, there is no evidence of an endocrine potential of deltamethrin.

No concerns over issues of endocrine disruption were raised neither during deltamethrin review by the Joint meeting on Pesticide Residues (JMPR) in 2001 nor within the EU regulatory process for inclusion of deltamethrin in Annex I of Directive 91/414/EEC.

CA 5.9 Medical data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Occupational medical experiences with Deltamethrin

In-company experience

Chemical name:

(IUPAC): (S)-alpha-cyano-m-phenoxybenzyl-(1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CAS: 52918-63-5, (S)-cyano(3-phenoxyphenyl)methyl (1R,3R)- 3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylate

Physical state: white powder

Processing plant: [redacted] India

Number of employees handling product: 28 workers plus 21 helpers

Production period: 2004 to 2013

Amount produced: in the last 10 years 263.5 to 328.85 metric tons/year

Personal safety measures: Work clothing, rubber boots, helmet, rubber chemical protection gloves, goggles, for some job tasks face shields, dusk mask. For open handling and potential product contact depending on risk assessment full face mask with ABEDK filter, or PVC moonsuit with respirator (self-contained breathing air), full-face mask with ABEK filter for respiratory protection.

In-company experience:
No unusual occurrences or complaints.

Occupational Medical Experiences

No. of workers exposed:	28 workers plus 21 helpers
Medical examinations:	History and full physical examination including simple neurological tests (reflexes, sensibility, coordination)

Commenced in:	2004
Examination intervals:	Quarterly for workers, annually for helpers
Laboratory examinations:	FBC, urine examination, FBS, lipid profile, liver enzymes, urea, creatinin,
Technical examinations	Chest X-ray, ECG, spirometry, vision testing, audiometry, if applicable (breathing protection, noise exposures, fork lift driving)
Other technical details:	n/a

Medical assessment:

Occupational medical surveillance of workers exposed to Deltamethrin performed since 10 years on a routine basis, not directly related to exposures, did not reveal any unwanted effects in the workers. The examinations included the above laboratory parameters and clinical and technical examinations.

During the production period since 2005 no accidents with Deltamethrin itself occurred in the workers (one scalding), and no consultations of the Medical Department due to work or contact with Deltamethrin were required.

CA 5.9.2 Data collected on humans

Data on human cases brought to the knowledge of the producer are collected in a database. The total of cases since 2004 was 1428 – many of them enquiries regarding asymptomatic patients only, others on definitely unrelated symptoms like infections or clearly due to other causes, and most of them with minor symptoms only.

Cases have been assessed in detail for the years 2012 and 2013.
There were a total of 74 cases, which could be evaluated. The symptoms reported consisted primarily of “Cold Burn” and airway irritation, in some cases prompting asthmatic reactions. Both are expected after dermal contact and inhalation in sensitive individuals. Some possibly allergic reactions have been reported with hives, rash or laryngeal swelling. It is unclear, whether deltamethrin or formulation ingredients would have been causal.
In some cases of oral ingestion in infants and suicidal attempts in teenagers and adults, mainly from China, only nausea and vomiting were reported, making these cases “minor”, too.
All cases could be assessed as minor or enquiries only. No severe symptoms or fatalities have been reported.
172 more cases were reported only from China as information only by Poison Control Centers without data, so that no assessment could be done. The same holds true for 4 cases from Colombia, in which no symptoms were reported in alleged poisonings.
For the time from 2006 to 2011 comparable annual cases numbers have been registered. There was one case of a severe anaphylactic reaction in a toddler exposed to residues of a freshly sprayed deltamethrin product. Other than that no cases related to products with deltamethrin had to be classified as severe or fatal, all were minor or enquiries only.

Before 2006 few cases have been recorded, again only minor in severity.

CA 5.9.3 Direct observations

Reports on Deltamethrin human poisoning cases in medical literature:

Since the last report in 2002 single cases of Deltamethrin poisoning have been reported in medical literature as have been overviews of pyrethroid poisoning case series also including Deltamethrin cases without detailed specification.

The symptoms reported from single cases were as follows:

Patient	Route	Dose	Signs and symptoms	Outcome	Reference(s)
21y, m	Oral	500 mg + 20ml benzene	Dyspnea, flushm then coma, persistentathetosis	Survived with sequelae	[redacted]; 2007, M-476525-01-2
1,5y, f	Oral	unknown	Vomiting, cough, drowsiness, fever. Resp.distress after 1 week	Survived	[redacted]; 2008, M-476804-01-1
16y, f	Oral	250 mg	Convulsions, cerebral edema	Survived	[redacted]; 2009, M-476294-01-1
24y, f	Oral	Unknown	Convulsions, coma	Survived	[redacted]; 2009, M-476295-01-1
53y, m	Oral	Unknown	Confusion, coma, apnea, cardiac arrest	Fatal*	[redacted]; 2009, M-476302-01-1
32y, f	Oral	10 g	Irritability, muscle cramps and fasciculations, burning sensation, loss of sensation in feet and arms, dyspnea, tachycardia, salivation	Survived	[redacted]; 2010, M-476303-01-1

*: Fatal outcome related to 95% hydrocarbons in formulation by the authors

CA 5.9.4 Epidemiological studies

A total of 113 poisoning cases with Deltamethrin including 1 fatality have been reported from China in 2006 ([redacted]; 2008; M-462640-01-1).

An overview of pyrethroid (not specifically Deltamethrin) toxicity has been given in the publication [redacted]; 2005; M-462619-01-1:

Pyrethroid poisoning cases have been few and until 2005 less than 10 deaths have been reported from ingestion or occupational exposure. Paresthesia, called “Cold Burn”, is the typical symptoms from dermal contact, while ingestion causes sore throat, nausea, vomiting and abdominal cramps. Dizziness, headache, fatigue, palpitations, chest tightness, blurred vision and in severe cases coma and convulsions may ensue.

Further case series in literature are more than 10 years old and have already been reported.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

In cases of contact to pyrethroids the first sign of exposure is a specific paresthesia/irritation, often described as "cold burn". This may appear immediately or shortly after contact to the substance, may last up to 24 (rarely to 48) hours, and often is reported to be worsened by warmth (e.g. showering). This "cold burn" is due to a stimulation of free nerve endings, and is dependent on concentration, not on dose. It is strictly a local symptom only and not a symptom of a systemic poisoning.
The irritation can occur both on the skin and on the mucous membranes of the airways. In the latter case in sensible individuals an asthma-like unspecific response can be triggered.
Metabolites of Deltamethrin can be measured in urine. The unspecific pyrethroid metabolite 3-phenoxybenzoic acid can be formed from most other pyrethroids, too, while the metabolite cis-Br$_2$CA is specific for deltamethrin.
([redacted]; 2009; Fourth national report on human exposure to environmental chemicals; M-475775-01-1)

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First Aid:

- Remove patient from exposure/terminate exposure under self-protection (e.g.long gloves)
- Thorough skin decontamination with copious amounts water and soap/detergent, as pyrethroids are very little soluble in plain water
  Note: Warm water may increase the subjective severity of the irritation/paresthesia, which is not a sign of systemic poisoning.

Treatment:

Gastric lavage should be considered in cases of significant ingestions within the first (2) hour(s); however, the application of activated charcoal and sodium sulphate is always advisable in significant ingestions.
There is no specific antidote for pyrethroids, any treatment thus can only be symptomatic.
Reports from the USA seem to indicate a positive effect of vitamin-E-containing oils on the irritation/paresthesia, however, there is no real proof of this. The skin application of oils or lotions containing vitamin E may be considered. The skin irritation may be painful and require the application of analgetics.
Anaesthetic eyedrops may be required in case of eye contamination after flushing.
In cases of severe ingestions cardiac and respiratory function should be monitored.
In case of convulsions diazepam is the anticonvulsant of choice. Thus seizure management should follow standard practice using benzodiazepines (with oxygen and airway protection), if insufficiently effective followed by Phenobarbital infusion as required for status epilepticus.

A suggested regimen would be:
Start with 10 to 30 mg diazepam by intravenous injection according to body weight, for children pro rata. This dose is to be repeated every 10 to 30 minutes according to the patient's response.
If salivation is very strong a single dose of atropine may be of help: 0.6-1.2 mg for adults, 0.02 mg/kg body weight for children.

Contraindications:
Adrenergic compounds (except for CRP) and high dose atropine.

Pyrethroid poisoning should not be confused with carbamate or organophosphate poisoning.

When poisoning is survived, recovery is spontaneous and without sequelae.

CA 5.9.7 Expected effects of poisoning

Signs and Symptoms of Poisoning:

In cases of contact to pyrethroids the first sign of exposure is a specific paresthesia/irritation, often described as "cold burn". This may appear immediately or shortly after contact to the substance, may last up to 24 (rarely to 48) hours, and often is reported to be worsened by warmth (e.g. showering).
This "cold burn" is due to a stimulation of free nerve endings, and is dependant on concentration, not on dose. It is strictly a local symptom only and not a symptom of a general poisoning.
The irritation can occur both on the skin and on the mucous membranes of the airways. In the latter case in sensible individuals an asthma-like unspecific response can be triggered.
In case of severe intoxications alpha-cyano pyrethroids may cause the following signs and symptoms as seen in animal experiments and suicidal poisoning cases:

Organ (system)	Signs/symptoms	Remarks
Skin	Paresthesia/irritation ("cold burn")	Local only
Mucous membranes	Irritation, cough, sneezing	Local only
Lung	Chest tightness, airway hyperreaction, "asthma", lung edema	
Heart/circulation	Tachycardia, hypotension, palpitations	
Gastrointestinal tract	Nausea, vomiting, diarrhoea, abdominal pain, salivation	
Central Nervous System	dizziness, blurred vision, headache, listnessless, anorexia, somnolence, coma, seizures/convulsions; tremor, ataxia, choreoathetosis (observed in animals only); muscle fasciculations	

No late effects of pyrethroid poisoning have been described in the scientific literature.

Proposal for new endpoints

The following human reference values have been adopted during the first inclusion of deltamethrin in Annex I:

- Acceptable Daily Intake (ADI): 0.01 mg/kg bw/d based on the one-year or 90-day dog NOAEL and a 100X uncertainty factor.
- Acute Reference Dose (ARfD): 0.01 mg/kg bw as for the ADI.
- Acceptable Operator Exposure Level (AOEL): 0.0075 mg/kg bw/d on the basis of one-year dog or 90-day NOAEL and a 100X uncertainty factor and adjustement for gastrointestinal absorption of 75%

However, Bayer CropScience would like to argue against the ARfD set during the last Annex I inclusion review and propose and ARfD of 0.05 mg/kg based on deltamethrin acute neurotoxicity study in the rat with a NOAEL of 5 mg/kg and a 100-fold safety factor, as established and re-discussed by the WHO/JMPR in 2000 and 2006.

In 2002, during the review of deltamethrin under Dir. 91/414, the former Rapporteur Member State (RMS) Sweden has reviewed deltamethrin acute neurotoxicity study and concluded that it was not acceptable due to the absence of data on food consumption and primarily no investigation of nervous tissue from the intermediate and low dose groups ([redacted]; 1998; M-152563-01-1). The RMS considered that there was an increased incidence of digestion chambers in peripheral nerves with or without axonal degeneration in two out of ten animals receiving deltamethrin at a dose of 50 mg/kg bw compared with none in the control group. In the deltamethrin Review Report (6504/VI/99-final; 17 October 2002), the EU Commission concluded that deltamethrin Acute Reference Dose (ARfD) was 0.01 mg/kg bw based on the neurotoxic effects of this active ingredient and the No Adverse Effect Levels (NOAEL) of 1 mg/kg bw/day of a 1-year and a 90-day study in the dog and a safety factor of 100.

In contrast, in 2000 the Joint FAO/WHO Meeting on Pesticides Residues (JMPR) has set an ARfD for deltamethrin of 0.05 mg/kg bw on the basis of deltamethrin acute neurotoxicity study and a NOAEL of 5 mg/kg bw/day and the application of a 100-fold safety factor. Effects noted at the LOAEL of 15 mg/kg bw/day included salivation, reduced motility in an open-field test, and soiled fur. The JMPR concluded that microscopic examination of perfused tissues including sciatic, tibial and peroneal nerves from rats treated at 50 mg/kg bw/day revealed no treatment-related neuropathological lesions (JMPR, 2000).

At the thirty-eighth session of the CCPR, the EU delegation has raised concerns regarding the ARfD for deltamethrin established by the JMPR in 2000 and asked the JMPR to review the basis for the ARfD established.

The 2006 JMPR therefore re-considered deltamethrin acute neurotoxicity study, historical control data on nerve lesions from neurotoxicity studies ([redacted]; 2001; M-240464-01-1) and a recent publication showing an ED30 of 2.5 mg/kg bw and a threshold dose of 1 mg/kg bw for reduced motor activity in rats exposed to deltamethrin by gavage ([redacted]; 2005; M-476568-01-1, referenced as KCA 5.8 /52). Considering the threshold dose of 1 mg/kg bw by gavage as a Cmax effect and applying a compound-specific assessment factor of 25, the JMPR calculated an ARfD of 0.04 mg/kg bw, thus confirming the ARfD of 0.05 mg/kg bw set in 2000.

Non relevance of the sub-chronic dog studies to set deltamethrin ARfD:
In the deltamethrin 91/414 Monograph, Addendum B, former RMS Sweden has stated that *"The NOAEL for deltamethrin used for risk assessment is 1 mg/kg bw/day based on neurotoxicological signs (unsteadiness of the gait, chewing/scratching of the extremities, tremor) noted in dogs at 10 mg/kg bw/day ([redacted] 1993)".*

Deltamethrin toxicity was evaluated in the Beagle dog in a 90-day study conducted by oral administration of the active ingredient (a.i.) in gelatine capsules at dose levels of 2, 10 and 50 mg/kg bw/day ([redacted]; 1991; M-149358-01-1). No mortality was reported and treatment related findings were confined to the 50 mg/kg bw/day dose group. The earliest signs of neurotoxicity were observed in week 1 of treatment then intermittently and included abnormal gait and head movements, tremors, salivation, vomiting, chewing and scratching of the extremities, hunched posture and quiet behavior.
In a subsequent 1-year study, Beagle dogs (4/sex/group) received deltamethrin by oral administration in capsules at dose levels of 1, 10 and 50 mg/kg bw/day ([redacted]; 1993; M-149298-01-1). No treatment related findings were reported at the lowest dose level. At the top dose level, all dogs exhibited clinical signs of neurotoxicity starting from week 1. At 10 mg/kg bw/day, clinical signs of neurotoxicity were seen in 3 females and one male. The earliest sign of neurotoxicity was observed on two occasions in one female dog four hours post- dose in week 3 of treatment and consisted of abnormal hind gait. In week 4, one female displayed stiff hind gait on one occasion 5 hours post dosing. The clinical signs of neurotoxicity reported in another female and a male were observed after 12 and 24-32 weeks of treatment, respectively.
Based on these findings, while the clinical signs of neurotoxicity reported are treatment related, the period of dosing versus expression of effects does not support the use of this data for an acute risk assessment. This is further supported by the 90-day study ([redacted]; 1991; M-149358-01-1).
In 2002, the JMPR has defined the ARfD as followed: *"The ARfD of a chemical is an estimate of the amount of a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 hours or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation"* (JMPR, 2002).
In their review paper for the guidance on the setting of ARfD for pesticides, [redacted] have described a stepwise process that should consist of a) a review of the total database of the a.i. to identify any potential toxic effect that could result from a single exposure; b) selection of the most relevant endpoint for a single exposure; c) selection of the most appropriate study in which this/these endpoint(s) have been investigated; d) identification of the NOAEL(s) for this/these endpoint(s) e) selection of an appropriate safety factor for derivation of the ARfD ([redacted] 2005; M-476565-01-1, referenced as KCA 5.8 /53).

Owing to deltamethrin toxic properties, the most relevant endpoints for setting an ARfD are the neurotoxic signs. However, the 90-day and 1-year dog studies are not relevant for deriving deltamethrin ARfD since the neurotoxic effects were expressed after a repeated dietary exposure of 3

weeks and more, and therefore do not reflect a realistic acute exposure scenario in humans. Therefore, deltamethrin acute neurotoxicity study in the rat is the most relevant study and endpoint to derive an ARfD.

Relevance of the acute neurotoxicity for deriving deltamethrin ARfD:

Deltamethrin potential for acute neurotoxicity was assessed in rats (12/sex/group) by a single gavage administration at dose levels of 0, 5, 15 and 50 mg/kg bw in corn oil (5 ml/kg) ([redacted] 1998; M-152563-01-1). The viability, clinical signs and body weights were recorded. Functional observational battery (FOB) and locomotor activity data were recorded prior treatment, at the time of peak effect (approximately 3 hours post dosing) and on days 7 and 14 for all animals. All surviving animals were sacrificed on study day 15 and perfused *in situ*. Whole brain weights and brain dimensions were recorded. A neuropathological evaluation was performed on 5 animals/sex in the control and 50 mg/kg bw groups. A necropsy was performed on all animals that died during the study. One male and one female died at the top dose level following 3.5 to 4 hours post dosing. Treatment related findings were confined to the top dose group and included gait alterations in two males and two females on days 1 and/or 2, yellow staining on the abdomen and/or urogenital area in three males and seven females on days 1, 2 and/or 3, and tan staining around the mouth in two males on days 1 and/or 2 and in one female on day 1. During the FOB, the following observations were made:

• 50 mg/kg bw group: In general, the responses occurred approximately 3 hours post dosing and were transient in nature. No signs of toxicity were apparent in these same animals during the FOB evaluations on study days 7 and 14 or the daily clinical observations after study day 3. Altered posture, tonic and clonic convulsions, tremors, alterations in biting and palpebral closure were observed during the home cage observations, and one female exhibited soft feces. Alterations in ease of removal from the cage, ease of handling, lacrimation, salivation and fur appearance were reported during the handling observations. Chromodacryorrhea and altered palpebral closure were observed in one male and female, respectively. During the open field observations, increased time to first step, impaired mobility and gait, clonic and tonic convulsions, tremors, decreased arousal, bizarre and/or stereotypic behavior (writhing) and decreased rearing, grooming and urination were observed. Sensory observations included altered approach, touch, startle and tail pinch responses, olfactory orientation, forelimb and hindlimb extension and air righting reflex. During the neuromuscular observations, reduced forelimb and hindlimb grip strength, impaired rotarod performance and altered hindlimb footsplay (males only) were noted. Physiological observations indicated increased group mean catalepsy values and decreased group mean body temperatures.

• 15 mq/kg bw group: On day zero, slight salivation in one male and slightly soiled fur in one female were noted during handling observations. One male had slightly impaired mobility during the open field observations.

• 5 mg/kg bw group: No treatment related findings were reported.

Increased mean ambulatory and total motor activity counts were apparent on day zero in males treated at 50 mg/kg bw. No treatment related effects were seen in brain weights or brain dimensions for perfused animals and no treatment-related neuropathological lesions were observed upon microscopic examination of 5 animals/sex in the 50 mg/kg group. Based on these results, the NOAEL for neurotoxicity was 5 mg/kg bw.

Within the regulatory process for inclusion of deltamethrin in Annex 1 of Directive 91/414/EEC, the former RMS Sweden reviewed deltamethrin acute neurotoxicity study and expressed concerns regarding neuropathological changes noted in the sciatic and tibial or peroneal nerves of one male and

one female in the high dose group. Based on this review the RMS determined this study to be unacceptable due to the absence of further neuropathological examination of the mid and low dose groups and on the absence of data on food consumption.

Further historical control data of neuropathological observations were requested to the laboratory which performed the acute neurotoxicity study as mentioned in the following position paper:

Report:	KCA 5.7/03; [redacted]; 2001; M-240464-01-1
Title:	Aventis CropScience Response to RMS Review of the Acute Neurotoxicity Study
Report No.:	B003436
Document No.:	M-240464-01-1
Guideline(s):	--
Guideline deviation(s):	--
GLP/GEP:	**no**

The laboratory that performed the acute neurotoxicity study, [redacted] Ohio USA, has provided historical control data of neuropathological observations for the [redacted] rat in both the acute and subchronic neurotoxicity studies for deltamethrin and further explanation from the report pathologist, [redacted] concerning the frequency and significance of axonal degeneration and digestion chambers of the sciatic and peroneal nerves. These data clearly show that effects noted in the two high dose animals were within historical control range and that these types of lesions are relatively common and spontaneous in nature in the rat

In addition to the historical control data provided in the individual acute and subchronic neurotoxicity reports, [redacted] has provided further historical control data from other subchronic neurotoxicity studies. As indicated by [redacted] "digestion chambers" in peripheral nerves have often been found as spontaneous lesions in the rat and an incidence of digestion chambers in the sciatic nerve of one to two animals in a control group is not uncommon.

Results from validated and standardised regulatory studies including the chronic toxicity/oncogenicity and subchronic neurotoxicity studies for deltamethrin gave no indication of treatment-related neuropathological change in the central or peripheral nervous tissue in rats.

Based on this information, Bayer CropScience believes that the neurotoxicity and neuropathological potential of deltamethrin has been adequately tested, and that the effects seen in one male and one female rat in the high dose group in the deltamethrin acute neurotoxicity study were spontaneous in nature and not treatment-related. Therefore, histopathological evaluation of the low and mid dose animals was not necessary.

During its 2006 review for the setting of deltamethrin ARfD, the JMPR has concurred with this position and concluded that *"Peripheral nerve oedema was considered to be a phenomenon that occurred only at high dose, and that other end-point evaluated in the study were judged adequate to identify a NOAEL"*.

Bayer CropScience therefore considers that the acute neurotoxicity study provides a NOAEL based on the most appropriate endpoint for setting deltamethrin ARfD. A conventional safety factor of 100 to account for inter-individual and inter-species variability is used to derive deltamethrin ARfD:

$$\frac{\text{NOAEL from the acute neurotoxicity study}}{\text{Safety factor}} = \frac{5 \text{ mg/kg bw}}{100} = 0.05 \text{ mg/kg bw}$$

The neonatal rat, due to metabolic limitations, is a conservative animal model for predicting potential adverse effects in infants and children. Data from deltamethrin DNT study support the conclusion that there is no concern for sensitivity of children and infants, and additional uncertainty factors for this concern are not required.