Renewal Assessment Report

beta-cyfluthrin

Volume 3 – B.6 Toxicology and metabolism data

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Rapporteur Member State: Germany

Co-Rapporteur Member State: Hungary

Version history

When	What

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B.6 Toxicology and metabolism data

Original DAR (1996):

Beta-cyfluthrin is a pyrethroid, consisting of 2 diastereoisomeric pairs, which are the biologically active isomers of cyfluthrin. They are contained in cyfluthrin at a percentage of about 40 %.
Due to the fact that in all studies on the metabolism with cyfluthrin these isomers were contained and therefore also tested, the full package of data on the metabolism of cyfluthrin can be regarded as being representative also for beta-cyfluthrin. Also a great part of toxicological studies were not performed with beta-cyfluthrin. However, the data obtained for cyfluthrin allow a true assessment of the toxicological properties of beta-cyfluthrin. For single studies or chapters referring to cyfluthrin the substance name is mentioned in the headline.

Re-evaluation by the RMS (2015):

For the AIR 3 re-assessment the old assessment from the original DAR (1996, ASB2010-10436) and the assessment in the subsequent addendum 1 (2002, ASB2014-9599) were integrated into one document. The section headings were adapted to current standards.

The applicant submitted new studies to support the re-approval of the active substance (ADME, acute toxicity, genotoxicity, short-term/repeat dose toxicity, reproductive toxicity/teratogenicity, neurotoxicity, and medical data). Besides these new submitted studies all previously available studies were re-evaluated and current criteria were applied. For the old study summaries, when considered necessary, the wording was changed for better comprehension and/or data tables were added. The results of the re-evaluation and evaluation for new submitted studies are given below each study summary.

Summaries regarding the individual endpoints, the derived reference values and proposals for classification and labelling are included in Volume 1, section 2.6 and 2.10.

A publication listed in the reference list of the applicant (Pesticide residues in food, FAO and WHO, 1987, TOX9401964) presents a summary about toxicological data of cyfluthrin (FCR 1272). However, it reports no further results compared to the studies presented in the RAR.

The applicant submitted a document about a literature search in the context of renewal of beta-cyfluthrin (ASB2014-7922). The literature search covered a period of ten years (January 2004 – November 2013) and is generally based on the guidance document of EFSA (Journal 2011; 9(2): 2092). A number of 15 databases were used to retrieve records for beta-cyfluthrin, its diastereomers or some of its metabolites. For the parent compound 2020 literature references and for the metabolites further 111 literature references were recorded (duplicates excluded).
After literature search the references were screened by experts according to relevance for human health effects, environmental fate and behaviour as well as ecotoxicology. In total 159 references were left. These remaining references were full-text reviewed. Finally, 5 studies were evaluated as relevant and therefore included in the dossier. However, none of these 5 studies was related to human health effects.
From the perspective of the RMS the selection of relevant studies concerning human health effects seems arbitrary. The rationales given for not including studies of this endpoint in the dossier are not in line with the requirements of a systematic review.
Taken together, the literature search strategy seems to be plausible whereas the study selection process is rather vague.

A literature search generally based on the guidance document of EFSA was conducted by the RMS (date: January 2015; keyword: *Cyfluthrin* or 68359-37-5; last 10 years).
The databases selected and the numbers of articles retrieved are summed up in the following table.

Table B.6 -1: Overview on the literature search conducted by the RMS

PubMed	259
Scopus	1367
ToxLine	15

The following criteria for inclusion and exclusion were applied for reduction of retrieved articles:

Table B.6 -2: Overview on the applied inclusion and exclusion criteria

Database	Criteria - inclusion	Criteria - exclusion
PubMed	tox* epidemiolog* human* medical* clinical* "acute tox*"	analyt* residue* ecotox* environm* aquat*
Scopus	tox* epidemiolog* human* clinical* medical* acut*	analyt* residues* ecotox* aquat* environm* malaria* leishmanios* efficac* method* resist* assay*
ToxLine	epidemiol* human* clinical* medical* acut*	PubMed pubdart analyt* ecotox* residues* aquat* environm* malaria* leishman* efficac* method* resist* assay* mosquit*

A number of 69, 42 and 2 references were left for PubMed, Scopus and ToxLine database, respectively.
The titles and abstracts of these remaining references were screened for potential relevance for the toxicological assessment. The selected articles are described in the respective sections of the RAR.

B.6.1 Absorption, distribution, metabolism and excretion in mammals

B.6.1.1.1 Absorption, distribution, metabolism and excretion by oral route

Four new studies on toxicokinetics have been generated since Annex-I inclusion of cyfluthrin/beta-cyfluthrin and the publication of the addendum 1 (2002, ASB2014-9599). Except the new studies, all studies here have been previously submitted. For the renewal process they were again evaluated.
A literature search for the Renewal Assessment Report (RAR) including publications from the last 10 years was performed by the RMS. The publications were considered as supplemental information.

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October 1996 (ASB2010-10436):

Data point:	KCA 5.1.2
Report:	[redacted], 1981, RIP9400855: Thiocyanate excretion in rats' urine after intraperitoneal administration of FCR 1272 and decamethrin in comparable doses and after exposure to defined FCR 1272 concentrations in the inhalation air. Report No.: PH 10130, (August 17, 1981); [redacted]
Guideline(s):	No guideline available
Deviations:	Not applicable
GLP:	GLP was not compulsory at the time the study was performed)
Acceptability:	Acceptable

(Dates of exp. work: June, 1982)

Materials and methods:

Test substance: Cyfluthrin, batch number: 816 070 017, isomer ratio: I 24.9 %, II 17.9 %, III 30.0 %, IV 22.2 %, purity: 95 %. Decamethrin purity: 99.2 %.
Animal species: Wistar albino rats (BOR:WISW (SPFCpb), source: [redacted]) 10 males or females/group, weight 160-200 g. Administration: Groups of ten rats each received 0, 1, 5, 10, 15 mg/kg bw FCR 1272 and 5, 10, 15 and 20 mg decamethrin/kg body weight intraperitoneal (only males). The animals were then placed immediately in metabolism cages and the urine was collected for up to three days after start of study.
Groups of ten male and ten female rats per concentration were exposed to FCR 1272 aerosols at 0, 59, 93, 180 mg/m^3 air for four hours. At the end of exposure the animals were placed in metabolism cages. The urine collected 0-20, 20-44, 44-68 and 68-92 hours after end of study was examined for its thiocyanate content.

Results and discussions:

Doses of 1-10 mg FCR 1272/kg body weight and of 5-20 mg decamethrin/kg body weight were tolerated by the rats without clinical symptoms and mortalities. At a dose of 15 mg FCR 1272/kg body weight, three of 10 treated rats died with the symptoms typical for FCR 1272. Despite great individual fluctuations, it was observed that the animals excreted thiocyanate in relation to dose. Thiocyanate was detectable in the urine for up to 72 hours. A total of approx. 24-42 % of the FCR 1272 administered was excreted through the kidneys in the form of thiocyanate. In contrast only approx. 6-10 % of the active ingredient administered was excreted as thiocyanate with the urine after intraperitoneal administration of 5, 10, 15 and 20 mg decamethrin/kg body weight.
All concentrations of the inhalation study were toxic to the rats, showing symptoms typical for FCR 127 and 5 female rats of the highest dose group died. If a respiration minute volume of 120 ml is used

as a base for the animals, 4-6 % of the active ingredient intake for the males and 2-6 % for the females was excreted in the form of thiocyanate.

Re-evaluation by the RMS (2015):

The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph (1996, ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.1.1
Report:	[redacted], 1982, RIP9400862: Biotransformation of [F-phenyl-UL-^{14}C]cyfluthrin; characterisation and preliminary identification of the metabolites. Report No.: PF 1632; (January 21, 1982); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compulsory.
Deviations:	Not applicable
GLP:	No (When the study was performed, GLP was not compulsory)
Acceptability:	Supplemental

(Dates of exp. work: not given)

Materials and methods:

Test substance: [F-phenyl-UL-^{14}C]-cyfluthrin, batch number: not given, cis/trans isomer ratio: 42/58 %, radiochemical purity: 98 %, 63.5 µCi/mg.
Animal species: Sprague Dawley rats (source: [redacted]), 2 males/group, weight approx. 200 g).
The labelled parent compound was administered orally at a dose of 10 mg [^{14} C] Cyfluthrin per kg body weight (as an emulsion in physiological saline solution containing detergent). The urine samples were collected at intervals of 0-8 h and 8-24 h after administration. The radioactivity of the urine samples, extracts and solutions was determined by liquid scintillation spectrometry (LS spectrometry).

Results and discussions:

By 24 hours after oral administration of 10 mg [F-phenyl-UL-^{14}C]-cyfluthrin per kg body weight to male rats, most of the radioactivity (>65 %) was eliminated in the urine. The metabolites in the urine of male rats 8 hours after oral application of 10 mg [F-phenyl-UL-^{14}C]-cyfluthrin per kg body weight, were characterised and tentatively identified as conjugates of preponderantly 4'-hydroxy-3-phenoxy-4-fluorobenzoic acid (approx. 50 % of the renal radioactivity), and 3-phenoxy-4-fluorobenzoic acid (approx. 40 %).

Re-evaluation by the RMS (2015):

The study is now considered to be supplemental since no information on the batch number and on the dates of the experimental work are given. In the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered to be acceptable.

Data point:	KCA 5.1.1
Report:	[redacted] 1982, RIP9400865: Comparative study of rats on absorption of FCR 1272 after single oral administration in polyethylene glycol 400 or Cremophor EL/water as formulation vehicle. Report No.: PH 10715 (March 10, 1982); [redacted]

Guideline(s):	At the time the study was performed, no particular method was compulsory.
Deviations:	Not applicable
GLP:	No (When the study was performed, GLP was not compulsory)
Acceptability:	Not acceptable

(Dates of exp. work: not given).

Materials and methods:

Test substance: Cyfluthrin (FCR 1272), batch number: 816170019, isomer ratio: I 26.6 %, II 19.1 %, III 33.7 %, IV 20.6 %, purity not reported.
Animal species: Wistar albino rats (BOR:WISW SPF 68 Han, source: [redacted]) 14 males per group/ 4 males (control-group), weight 160-170 g.
Administration:
1st group: 10 mg/kg bw cyfluthrin (one single oral dose; administration volume 5 ml/kg bw) in the form of a 0.2 % solution in lutrol (polyethylene glycol 400).
2nd group: 10 mg/kg bw cyfluthrin (one single oral dose; administration volume 10 ml/kg bw) in the form of a 0.1 % emulsion in Cremophor EL/distilled water.
Control group: Four male unfed rats each received the solvents only.
0.5, 1, 2, 4, 6, 16, and 24 hours after administration, two rats per group were sacrificed by ether anaesthesia. Blood was taken via heart puncture. The stomachs were removed and opened. Cyfluthrin was determined in the blood and in the stomach extracts.

Results and discussions:

Absorption was fast using Cremophor EL/distilled water as formulant, and the 4 FC 1272 enantiomers were detected in the blood 30 min after administration. Maximum figures were reached after 1 hour. At this point the animals showed first signs of toxic symptoms typical for cyfluthrin. All animals survived and the symptoms had disappeared after 2 hours.
Using cyfluthrin in polyethylene glycol 400 the enantiomers could not be detected in the blood until 4 hours after administration. Maximum figures were reached after 6 hours, but these figures only reached about 1/5 of the maximum figures obtained after 1 hour for the animals treated with cyfluthrin in Cremophor EL/distilled water. Toxic symptoms were not observed.
Considerably higher amounts of cyfluthrin were found after 30 min up to 4 hours in the stomachs of animals treated with cyfluthrin in polyethylene glycol 400. After 30 min the concentrations were about 5 times higher than in the stomachs extracts of cyfluthrin in Cremophor EL/distilled water.
Cyfluthrin in Cremophor EL/distilled water is absorbed faster (maximum 1 hour) and more intensively than cyfluthrin in polyethylene glycol 400 (maximum 6 hours). The shift of the enantiomer ratio within the first few hours in favour of cis-enantiomers was observed in both groups. The results obtained demonstrate that the absorbability of cyfluthrin after oral administration depends largely on the polarity of the formulation vehicle. The toxicity of cyfluthrin in Cremophor EL/distilled water is caused by faster and higher absorption.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable since no information on the purity of the test substance and on the dates of the experimental work are given. In the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered to be acceptable.

The applicant disagreed with this decision and stated that although the study was not conducted to current guidelines, it delivers scientifically sound and significant information about the influence of the vehicle on the absorption of cyfluthrin and thus the study should be regarded as supportive information.
However, the conclusion by the RMS that the study is considered not acceptable remains. It is noted,

that this decision has no influence on the oral absorption value and on the reference values.

Data point:	KCA 5.1.1
Report:	[redacted], 1983, RIP9400866: Fluorophenyl-UL-^{14}C cyfluthrin (FCR 1272) biokinetic study in rats. Report No.: PH 11575(F), (February 18, 1983); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compulsory (the study partly complies with the OECD Guideline for Testing Chemicals No. 417, adopted July 2010)
Deviations:	None that compromised the validity of the study results (age of the rats are not given, absorption was determined on cannulated rats for dose group only (0.5 mg/kg bw), information on metabolism not given)
GLP:	No (When the study was performed, GLP was not compulsory)
Acceptability:	Acceptable

(Dates of exp. work: 1980 to 1981).

Materials and methods:

Test substance: cyfluthrin, batch number: 16003/79, cis/trans isomer ratio: 42/58, purity: 97.5 %, radiochemical purity: 98 %, 62 µCi/mg.
Animal species: Mura rats (SPRA:Han, source: [redacted]), 5/group, weight 190-210 g.
Administration: a) 0.5 mg/kg bw (single intravenous or intraduodenal, male rats), b) 0.5 mg/kg bw (single oral, male rats), c) 10 mg/kg bw (single oral dose, male rats), d) 0.5 mg/kg bw (single oral dose, female rats). The labelled substance was dissolved in toluene in a concentration of 8.4 mg/ml.
The rats used for excretory experiments and whole body autoradiography were housed individually in metabolism cages. Two-day experiments were performed on non-anesthetised, non-fasted male rats in which a biliary cannula had been surgically implanted on the day prior to administration.
The animals were narcotised with carbon dioxide and sacrificed. If blood was collected, it was separated into plasma and erythrocytes by centrifuging. The organs and tissues removed during necropsy were weighed while fresh and after freeze-drying mechanically pulverised.
The amounts of radioactivity excreted in the urine, faeces bile, and the expired gases, as well as the radiolabelled residues present in the body at the respective times of sacrifice are expressed in percent of administered radioactivity.

Results and discussions:

The mass balance (percent of excreted radioactivity in the bile, urine, faeces, expired air, body excluding / including gastrointestinal tract) 48 hours after administration was satisfactory (93.5-103.1 %).

Accumulation:
The kinetics of excretion, as well as the concentration curves in the individual tissues and organs, indicate that the small amount of residues (0.2 %) present in the body at the end of the study (10 day) do not accumulate, but continue to be eliminated.

Absorption:
Information on absorption can be obtained from the experiment on cannulated animals (0.5 mg/kg bw). An absorption index of about 90 % of the recovered amount is obtained from the total amounts of radioactivity determined in the urine (≈ 50 %), the bile (≈ 33 %), faeces (≈ 12 %) and in the body excluding gastrointestinal tract (≈ 0.5 %).

Elimination/Excretion of radioactivity:

The radioactivity is eliminated in the expired gases only to a small extent. 48 hours after the oral administration of 10 mg/kg bw, less than 0.001 % of the administered dose is expired.
The excretion is predominantly renal (renal/faecal: 2:1) for both routes of administration.
Within 2 days after oral administration of 0.5 and 10 mg/kg bw to male rats and 0.5 mg/kg bw to female rats, 59-74 % of the recovered amount is excreted renally, and 25 % to 39 % faecally (total >98 %).The amount of radioactivity excreted is proportional to the dose level tested and independent of the sex of the animals. The total amount of radiolabelled residues of the parent compound present in tissues (excluding gastrointestinal tract) is in the range of 1.1 % to 1.6 %.
After intravenous administration of 0.5 mg/kg bw to male rats, about 70 % is excreted renally and 24 % faecally. The total amount of radiolabelled residues of the parent compound present in tissues (excluding gastrointestinal tract) is 6 %.
After the intraduodenal administration of 0.5 mg/kg bw cyfluthrin to male rats, approximately on third of the recovered amount is excreted in the bile, more than 50 % within 2 hours, and more than 90 % within 6 hours after administration. The biliary excretion of radioactivity begins quickly after intraduodenal administration. The cannulated animals excrete about 54 % renally and 12 % faecally (enterohepatic circulation).

Distribution of radioactivity into tissues and organs:
After oral administration of 10 mg/kg bw to male rats, approximately 40 % of the administered radioactivity is present 1.5 hours after administration in the body, excluding gastrointestinal tract. Due to renal and biliary excretion, the value decreases to 20 % after 8 hours, 1.7 % after 2 days, and to 0.2 % after 10 days after administration.

Concentration of radioactivity in individual organs and tissues:
After oral administration of 10 mg/kg bw, at the time of maximum plasma level (1.5 hours after administration) values in the liver and in the kidneys were markedly higher in comparison to other organs/tissues. Parallel to the onset of excretion in urine and bile, a slow redistribution of radioactivity occurs into the fatty tissue. Two days after administration, the concentration in the renal fat is about 10 times higher than the mean concentration in the body, 10 days after administration it is about 20 times higher.

Concentration of radioactivity in the plasma:
Minimal radioactivity is found in the erythrocytes, which are 5 times lower than in the plasma level.

Re-evaluation by the RMS (2015):

Information on the metabolism is missing. The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.1.1
Report:	[redacted] 1983, RIP9400867: Biokinetic part of the general metabolism studies in the rat. Report No.: PH 11872(F), (June 9, 1983); [redacted]
Guideline(s):	The study was run according to EPA specifications (The experimental design is comparable to Directive 87/302/EEC, Part B. and partly complies with the OECD Guideline for Testing Chemicals No. 417, adopted July 2010)
Deviations:	None that compromised the validity of the study results
GLP:	No (When the study was performed, GLP was not compulsory)
Acceptability:	Acceptable

(Dates of exp. work: December 1980 to July 1981)

Materials and methods:

Test substance: [Fluorobenzene-UL-^{14}C]-cyfluthrin, batch number: 16003/79, cis/trans isomer ratio: 42/58, purity: 97.5 %, radiochemical purity: 98 %, 62 µCi/mg.
Animal species: Mura rats (SPRA, SPF 68 Han, source: [redacted]) 5 males or females/group, weight ca. 200 g).
Administration: Group A: 0.5 mg/kg bw (single intravenous and intraduodenal), Group B: 0.5 mg/kg bw (single oral), Group C: 0.5 mg/kg bw (a series of 14 daily oral doses of non-radioactive substance, followed by a single oral radioactive dose at the same level after 24 hours), Group D: 10 mg/kg bw (single oral). The labelled substance was dissolved in toluene in a concentration of 8.4 mg/ml.
The rats used for excretory experiments were housed individually in metabolism cages.
The biliary cannula had been surgically implanted on the day prior to administration. The animals were narcotised with carbon dioxide and sacrificed.
The collected blood was separated into plasma and erythrocytes by centrifuging. The organs and tissues removed during necropsy were weighed while fresh and after lyophilisation. Finally they were homogenised.

Results and discussions:

The mass balance (percent of excreted radioactivity in the bile, urine, faeces, expired air, body excluding / including gastrointestinal tract) 48 hours after administration was satisfactory (93-106 % in males; 93-101 % in females).

Accumulation:
The kinetics of excretion, as well as the concentration curves in the individual tissues and organs, indicate that the small amount of residues (0.06 % / 0.066 % in males / females after i.v. administration, 0.011 % / 0.013-0.016 % / 0.018 % in males / females after oral administration) present in the body exclusive gastrointestinal tract at sacrifice do not accumulate, but continue to be eliminated.

Absorption:
The comparison of the sum of the renally excreted and the radioactivity remaining in the body excluding the gastrointestinal tract at sacrifice (48 hours after administration), between intravenous and oral administration resulted in an absorption extent of nearly 100 % (males) and 90 % (females). Based on the sum of the radioactivity excreted via the bile fluid and urine plus the remaining radioactivity in the body excluding gastrointestinal tract the extent of absorption amounted to ca. 90 % of the given dose. This also holds for the multiple oral administration of the low dose to both sexes and the single oral administration of 10 mg/kg bw to male and female rats.
The absorption commences at about 13 min after administration and has an average half-life of ca. 34 min. No difference between sex, dose level or pre-treatment was noted.

Distribution:
The radioactivity of cyfluthrin was slowly distributed from the intravascular space to the tissues. After i.v. injection of 0.5 mg/kg bw the volume of distribution of the central compartment was ca. 17 % of the total body volume.

Excretion:
Within the period of 48 hours after i.v. injection and oral administration total excretion of both sexes amounted up to 94 % and 99 % of the radioactivity, respectively. The excretion ratio (urine/faeces) for the males was 2.9 and for the females 2.3. After oral administration males excreted about 2 to 3 times more via urine than via faeces. The renal excretion was rapid, 90 % were excreted within the first 24 hours. Within 48 hours via faeces 29 % of the retrieved dose was excreted in males and 35 % in females.
Approximately one third of the retrieved radioactivity was excreted via bile fluid during the first 2 hours and more than 90 % within the first 6 hours post application. Relating these results to the faecal excretion of intact rats following both routes of administration, it can be stated that at least one half of the faecally excreted radioactivity is due to an absorbed and the biliary eliminated amount. A part of the biliary radioactivity is subject to an enterohepatic circulation.

Concentration of radioactivity in individual organs and tissues:
In the sciatic nerve there was a relatively high concentration compared to the very low concentration in the brain.
In all cases under investigation the fat is the organ showing the highest relative concentration of cyfluthrin and/or its metabolites. It can be concluded that the fat is the target organ at least in rats reflecting the lipophilicity of cyfluthrin and/or its metabolites.

Re-evaluation by the RMS (2015):

The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.1.1
Report:	[redacted] 1983, RIP9400868: [Fluorobenzene-UL-^{14}C]-FCR 1272; [Fluorobenzene-UL-^{14}C]-cyfluthrin: Metabolism part of the general metabolism studies in the rat. Report No.: PF 2059, (September 14, 1983); [redacted] (see also Ecker, 1982, Klein et al., 1983)
Guideline(s):	The study was run according to EPA specifications (NTIS, USD Commerce, Pesticide Assessment Guidelines, Subdivision F, Nov. 1982). The method is compatible to Directive 87/302/EEC, Part B.
Deviations:	None that compromised the validity of the study results
GLP:	No (GLP was not compulsory at the time the study was performed)
Acceptability:	Acceptable

(Dates of exp. work: not given)

Materials and methods:

Test substance: [Fluorobenzene-UL-^{14}C]-cyfluthrin, batch number: 16003/79, cis/trans isomer ratio: 42/58, purity: 97.5 %, radiochemical purity: 98 %, 62 µCi/mg.
Animal species: Animal species: Mura rats (SPRA, SPF 68 Han, source: [redacted] 4 males or females/group, weight 200 g).
Administration: Group A: 0.5 mg/kg bw (single intravenous), Group B: 0.5 mg/kg bw (single oral), Group C: 0.5 mg/kg bw (a series of 14 daily oral doses of non-radioactive substance, followed by a single oral radioactive dose at the same level after 24 hours), Group D: 10 mg/kg bw (single oral).
The labelled substance was dissolved in toluene in a concentration of 8.4 mg/ml. The rats used for excretory experiments were housed individually in metabolism cages. Urine was collected 8, 24 and 48 hours after dosing, faeces were collected 24 and 48 hours after dosing. Metabolite distribution in urine and faeces was determined by Thin Layer Chromatography (TLC) analysis.

Results and discussions:

The excretion of radioactivity 48 hours after oral administration of the test compound exceeded 95 % of the totally (in excreta and body) recovered radioactivity for animals of either sex at low and high dose levels. The ratio of renal to faecal excretion ranged from 2:1 to 3:1 for males and 1.6:1 to 1.8:1 for females. This sex dependent excretion patter was considered to result from a lower degree of conjugation of the hydroxylated metabolite FCR3145 with a subsequent higher degree of faecal excretion of the non-conjugated compound by female rats of all dose groups.
The excretion of radioactivity 48 hours after i.v. administration of the test compound ranged from 93.7 % (males) to 90.5 % (females), the ratio of renal to faecal excretion being 2.6:1.
Metabolite M1 - conjugate of 4'-hydroxy-4-fluoro-3-phenoxybenzoic acid (OH-FPB acid) represented

51-52 % of the recovered radioactivity in dose groups A, B and C. Males excreted a higher amount of M1 than corresponding females.
Metabolite M2 was a minor metabolite representing 3 % or less of the recovered radioactivity and showed no significant differences between dose groups or sexes.
Metabolite FCR3145 - free 3-(-(4-hydroxyphenoxy)-4-fluorobenzoic acid accounted to 3-5 % of the recovered radioactivity in urine only of male animals of all dose groups. This metabolite complements for the inverse difference between the sexes concerning the metabolite M1.
Metabolite FCR3191 - free 3-phenoxy-4-fluorobenzoic acid represented approximately 10 % of the recovered radioactivity in males and females of dose groups A, B, and C (all low doses), whereas animals of dose group D generated an approx. double amount of this metabolite.
The unchanged parent compound FCR1272 accounted for approximately half of the faecally eliminated portion.
Identified metabolites comprise 65-72 % of the recovered radioactivity in the dose groups A and B (both single low dose) and approx. 82 % in the dose groups C (multiple low dose groups) and D (single high dose).

Re-evaluation by the RMS (2015):

The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered acceptable.

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

Data point:	KCA 5.1.1
Report:	[redacted], 2013, ASB2014-7716: Beta-cyfluthrin: Absorption, Distribution and Excretion of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin, formulated in Cremophor EL, in Male Rats After Single Oral Administration at One Dose Level. Company: [redacted] t No: R-30146, M-481053-01-1 Date: 2013-03-06 not published
Guideline(s):	OECD Guideline No. 417, July 2010
Deviations:	Metabolite profiling not performed
GLP:	Yes
Acceptability:	Acceptable

(Dates of exp. work: September 2012-November 2012)

Materials and methods:

Test substance: beta-cyfluthrin, radiolabeled test item: batch number: PNBC000623, isomer distribution: I: max. 2 %, II: 30-40 %, III: max. 3 %, IV: 57.0-67 %, purity: 99.3 %.
Animal species: Wistar rats (RccHan:WIST (SPF), source: [redacted]; 4 males /group, weight at dosing: 255 ± 3 g.
Administration: single oral dose of nominally 10 mg/kg bw beta-cyfluthrin in Cremophor EL via gavage. The target volume was 5 mL/kg bw.

Results and discussions:
One rat (number 2) had to be sacrificed for ethical reasons when the animal started convulsing.
All animals started showing signs of toxicity 1 hour after dosing. The symptoms were hypersalivation, piloerection, diarrhoea, and crouch position. Animals were symptom-free at around 10 hours after dosing. Thereafter, no unusual appearance/behaviour or signs of toxicity were observed.
Absorption and Excretion: The mass balance documenting the absorbed and excreted fractions is

shown in Table B.6.1-1. About 92 % of the administered dose was recovered from excreta within 48 h after dosing. The vast majority of excretion occurred within the first 24 h after dosing.
About 35 % of dose was recovered from faeces. Summing up the percent of dose found in urine, tissues and carcass, a minimum absorption of 60 % must have occurred. Only 3 % of the dose was present in the carcass at 48 h post dosing. A sufficiently high recovery of 95.3 % was achieved.

Table B.6.1-1: Mass balance of beta-cyfluthrin derived radioactivity in male rats

Mass Balance [% of dose]		
Urine		
	0-24 h	52.20
	24-48 h	4.70
	Subtotal	56.90
Faeces		
	0-24 h	26.15
	24-48 h	8.52
	Subtotal	34.67
Expired air		
	0-24 h	<0.01
	24-48 h	<0.01
	Subtotal	<0.01
Cage Wash		0.61
Total Excretion		92.18
Tissue Residues		
	Tissues	0.39
	GI contents	0.48
	Carcass	2.28
	Subtotal	3.14
Amount absorbed (minimum)*		60.18
Total Recovery		95.33

* The amount absorbed was calculated based on the radioactivity recovered in urine, cage wash, and residues in carcass and tissues.

Blood and plasma concentrations:
At 1 hour post dose, mean blood and plasma concentrations were 4.766 and 9.045 µg eq/g, respectively. At 48 hours post dose, mean concentrations decreased to 0.059 and 0.112 µg eq/g in blood and plasma, respectively. The higher concentrations found in plasma as compared to blood would suggest that test item related materials are mainly associated to the plasma fraction of the blood.

Distribution:
Mean concentrations of total radioactivity in tissues and organs are presented in Table B.6.1-2. Residual radioactivity at 48 hours post dosing could be detected in all tissues/organs analysed.
Highest concentrations of radioactivity were found in white fat, adrenals, kidneys, liver, lungs, pancreas, thyroids, gastrointestinal tract (without contents) and the remaining carcass. These tissues/organs had concentrations higher than in blood.
Tissues like brain, femur, heart, muscle, spleen, testis and thymus had concentrations lower than in blood.

Table B.6.1-2: Concentration of total radioactivity in tissues and organs

	Mean	SD	LOQ[1]
Dose [mg/kg bw]	9.99	0.13	
[µg equivalents per g]			
Adrenals	0.437	0.034	0.0042
Brain	0.004	0.001	0.0020
Fat (white)	1.355	0.518	0.0042
Femur	0.025	0.004	0.0008
Heart	0.021	0.003	0.0021
Kidneys	0.114	0.011	0.0021
Liver	0.183	0.026	0.0024
Lungs	0.067	0.024	0.0022
Muscle	0.014	0.007	0.0021
Pancreas	0.335	0.342	0.0021
Spleen	0.022	0.001	0.0021
Testis	0.017	0.002	0.0020
Thymus	0.035	0.006	0.0020
Thyroids	0.328	0.280	0.0200
GI-Tract	0.230	0.051	0.0022
Carcass	0.282	0.052	0.0004
Blood, 1 h	4.766	1.006	0.0025
Plasma, 1 h	9.045	1.947	0.0084
Blood, 48 h	0.059	0.005	0.0042
Plasma, 48 h	0.112	0.013	0.0042

[1] LOQ: Limit of Quantification

Conclusion:
After oral administration, [fluorophenyl-UL-^{14}C]-beta-cyfluthrin related material was rapidly excreted either via urine or faeces. Urinary excretion represented about 57 % of the dose and faecal excretion accounted for about 35 % of the dose. Within 48 hours, 92.18 % of the administered radioactivity was excreted with a total of 3.14 % of the residual radioactivity recovered in the gastro intestinal contents (0.48 %), tissues (0.39 %) and carcass (2.28 %). The amount absorbed was calculated based on the radioactivity recovered in urine, cage wash, and residues in carcass and tissues. At least 60.18 % of the dose was absorbed from the gastrointestinal tract into systemic circulation.
Information about radioactivity present in bile was not provided. Therefore, it cannot be assumed that the proportion excreted via faeces represents material which had undergone systemic absorption. A minimum oral absorption of 60 % can be assumed.

The study is considered acceptable under the conditions of the study and based on the information given in the report.

Data point:	KCA 5.1.1
Report:	[redacted], 2014, ASB2014-7717 Beta-Cyfluthrin: Absorption, Distribution, Excretion and Metabolism of [fluorophenyl-UL-^{14}C] Beta-Cyfluthrin, formulated in PEG400, in Male Rats After Single Oral Administration at One Dose Level. Company: Irvita Plant Protection Report No: R-30146a, M-481060-01-1
Guideline(s):	OECD Guideline No. 417, July 2010
Deviations:	None that compromised the validity of the study results
GLP:	Yes
Acceptability:	Acceptable

(Dates of exp. work: December 2012- December 2013)

Materials and methods:

Test substance: beta-cyfluthrin, radiolabeled test item: batch number: PNBC000623, isomer distribution: I: max. 2 %, II: 30-40 %, III: max. 3 %, IV: 57.0-67 %, purity: 99.3 %.
Animal species: Wistar rats (RccHan:WIST (SPF), source: [redacted]; 4 males /group, weight at dosing: 182 ± 5 g.
Administration: single oral dose of nominally 10 mg/kg bw beta-cyfluthrin in Cremophor EL via gavage. The target volume was 5 mL/kg bw.

Results and discussions:
No mortality occurred.
No unusual appearance/behaviour or signs of toxicity were observed.
The mass balance documenting the absorbed and excreted fractions is shown in Table B.6.1-3. About 97 % of the administered dose was recovered from excreta within 48 h after dosing. The vast majority of excretion occurred within the first 24 h after dosing.
About 30 % of dose was recovered from faeces, but without information about radioactivity present in bile, it cannot be proven that this proportion represents material which had undergone systemic absorption. Summing up the percentage of dose found in urine, cage wash, tissues and carcass, a minimum absorption of 68 % must have occurred. A sufficiently high recovery of 98.3 % was achieved.

Table B.6.1-3: Mass balance of beta-cyfluthrin derived radioactivity in male rats

Mass balance [% of dose]		
Urine		
	0-24 h	54.41
	24-48 h	9.85
	Subtotal	64.25
Faeces		
	0-24 h	21.01
	24-48 h	8.72
	Subtotal	29.74
Expired air		
	0-24 h	<0.01
	24-48 h	<0.01
	Subtotal	<0.01
Cage wash		2.69

Total excretion		96.69
Tissue residues		
	Tissues	0.45
	GI contents	0.22
	Carcass	0.89
	Subtotal	1.56
Amount absorbed*		68.29
Total recovery		98.25

* The amount absorbed was calculated based on the radioactivity recovered in urine, cage wash, and residues in carcass and tissues.

Blood and plasma concentrations:
At 6 hours post dose, mean blood and plasma concentrations were 5.546 and 10.641 µg eq/g, respectively. At 48 hours post dose, mean concentrations decreased to 0.045 and 0.076 µg eq/g in blood and plasma, respectively. The higher concentrations found in plasma compare to blood would suggest that test item related materials are mainly associated to the plasma fraction of the blood.

Distribution:
Mean concentrations of total radioactivity in tissues and organs are presented in Table B.6.1-4. Residual radioactivity at 48 hours post dosing could be detected in all tissues/organs analysed. Highest concentrations of residual radioactivity were found in white fat, in richly vascularised tissues: adrenals, kidneys, liver, lungs, pancreas, thyroids, the gastrointestinal tract (without contents) and the remaining carcass. These tissues/organs had concentrations equal or higher than the ones found in the blood. Contrary, tissues from brain, femur, heart, muscle, spleen, testis and thymus had concentrations lower than the ones found in blood samples.

Table B.6.1-4: Concentration of total radioactivity in tissues and organs[1]

	Mean	**SD**	**LOQ[2]**
Dose [mg/kg bw]	10.07	0.080	
[µg equivalents per g]			
Adrenals	0.157	0.106	0.0041
Brain	0.003	0.001	0.0021
Fat (white)	0.820	0.364	0.0041
Femur	0.014	0.013	0.0012
Heart	0.013	0.004	0.0021
Kidneys	0.092	0.024	0.0021
Liver	0.128	0.029	0.0023
Lungs	0.045	0.006	0.0022
Muscle	0.008	0.002	0.0021
Pancreas	0.046	0.042	0.0021
Spleen	0.010	0.004	0.0021
Testis	0.013	0.004	0.0019
Thymus	0.008	0.003	0.0019
Thyroids	0.060	0.027	0.0195
GI-Tract	0.119	0.064	0.0022

Carcass	0.108	0.034	0.0004
Blood, 6 h	5.546	1.320	0.0082
Plasma, 6 h	10.641	2.454	0.0082
Blood, 48 h	0.045	0.014	0.0021
Plasma, 48 h	0.076	0.022	0.0021

[1] Due to a low overall recovery for animal number 1, samples from this rat were not used.
[2] LOQ: Limit of Quantification

Urinary metabolite pattern:
The quantitative distribution of the urinary metabolite fractions is summarised in Table B.6.1-5 below (% of dose).

Table B.6.1-5: Urinary metabolite pattern

Urinary metabolite pattern [% of dose][1]			
Metabolite fraction	**Sampling time**		**Assignment**
	0-24 h	24-48 h	
U1	38.4	8.3	OH-FPB acid sulfate conjugate
U2	1.8	0.2	OH-FPB acid
U3	13.5	1.1	FPB acid
U4	0.4	0.1	beta-cyfluthrin
Σ	54.1	9.7	

[1] Due to a low overall recovery for animal number 1, urine samples from this rat were not used for metabolite profiling.

Faecal metabolite pattern:
The quantitative distribution of the faecal metabolite fractions is summarised in the next table (% of dose).

Table B.6.1-6: Faecal metabolite pattern

Faecal metabolite pattern [% of dose based on extraction recoveries][1]			
Metabolite fraction	**Sampling time**		**Assignment**
	0-24 h	24-48 h	
F1	0.18	0.19	
F2	0.90	0.65	OH-FPB acid
F3	0.43	0.44	FPB acid
F4	0.19	0.21	
F5	0.29	0.32	
F6	0.54	0.49	FPB-ald
F7	0.37	0.23	
F8	16.14	3.89	beta-cyfluthrin
Subtotal Non-extractable	19.05 0.48	6.41 0.65	
% dose recovered in matrix	21.01	8.72	

[1] Due to a low overall recovery for animal number 1, faeces samples from this rat were not used for metabolite profiling.

Plasma metabolite pattern:
The quantitative distribution of the plasma metabolite fractions is summarised in the next table (µg eq. beta-cyfluthrin/g).

Table B.6.1-7: Plasma metabolite pattern

Plasma metabolite pattern [µg eq. beta-cyfluthrin/g][1]		
Metabolite fraction	**Sampling time**	**Assignment**
	6 h	
P1	3.08	
P2	1.74	
P3	4.79	FPB acid
P4	0.15	beta-cyfluthrin
Extract Non-extractable	9.75 0.89	
Total µg eq. beta-cyfluthrin/g in sample	10.64	

[1] Due to a low overall recovery for animal number 1, plasma samples from this rat were not used for metabolite profiling.

Conclusion:

The amount of beta-cyfluthrin orally absorbed was calculated based on the radioactivity recovered in urine, cage wash (residues of dried urine), expired-air and residues in carcass and tissues. At least 68.29 % of the dose was orally absorbed from the gastrointestinal tract into systemic circulation.
The absorbed radioactivity was rapidly excreted either via urine or faeces. Urinary excretion represented 64.25 % of the dose. Faecal excretion accounted for 29.74 % of the dose. Information about radioactivity present in bile was not provided. Therefore, it cannot be assumed that the proportion excreted via faeces represents material which had undergone systemic absorption. A minimum oral absorption of 68 % can be assumed.
Within 48 hours, 96.69 % of the administered radioactivity was excreted with a total of 1.56 % of the residual radioactivity recovered in the gastro intestinal contents (0.22 %), tissues (0.45 %) and carcass (0.89 %). A total recovery of 98.25 % was achieved.
Beta-cyfluthrin was rapidly excreted, predominantly via urine. Tissue residues were low at 48 h post-dosing. Absorbed beta-cyfluthrin was extensively metabolised and the main urinary metabolite was the sulphate conjugate of OH-FPB-acid. Unchanged parent was the major test-related material found in faeces with traces amounts in urine and plasma. Other detected metabolites in urine and faeces were FPB-ald, FPB-acid and OH-FPB-acid.

The study is considered acceptable under the conditions of the study and based on the information given in the report.

Data point: KCA 5.1.1

Report: [redacted], 2013, ASB2014-7718
Beta-Cyfluthrin: Absorption, Distribution, Excretion and Metabolism of [cyclopropane-1-^{14}C]-beta-Cyfluthrin in Male and Female Rats After Single Oral Administration at Two Dose Levels. Company: [redacted]. Report No: R-30145, M-481047-01-1

Guideline(s): OECD Guideline No. 417, July 2010¸ EU B.36, 2008; OPPTS 870.7485

Deviations: None that compromised the validity of the study results

GLP: Yes

Acceptability: Acceptable

(Dates of exp. work: October 2012- September 2013)

Materials and methods:

Test substance: beta-cyfluthrin, radiolabeled test item: batch number: PNBC000623, isomer distribution: I: max. 2 %, II: 30-40 %, III: max. 3 %, IV: 57.0-67 %, purity 99.3 %.
Animal species: Wistar rats (RccHan:WIST (SPF), source: [redacted]; 4 males and 4 females /group, weight at dosing: males: 199 ± 17 g; females: 179 ± 3 g.
Administration: Four male and four female rats per dose level received either a single oral dose of 0.5 mg/kg bw (low dose) or 10 mg/kg bw (high dose) of beta-cyfluthrin in PEG400, via gavage. The target volume was 5 mL/kg bw.

Results and discussions:

No mortality occurred.
No unusual appearance/behaviour or signs of toxicity were observed in the low dose group.
For the high dose group the animals showed a slight piloerection and diarrhoea on the first day after application. Thereafter, no unusual appearance/behaviour or signs of toxicity were observed.

Absorption and excretion:
The mass balance documenting the absorbed and excreted fractions is shown in Table B.6.1-8. Over 90 % of the administrated dose was totally excreted within 48 hours, for both tested doses. And after 7 days almost the complete dose was excreted: 104.03 % (male) and 100.71 % (female) in the low-dosed group, and 105.19 % (male) and 108.42 % (female) in the high-dosed group of rats. Between 0.51 % and 0.65 % of the dose was found in the remaining carcass and tissues of rats seven days after dosing. Total recoveries were between 101.22 % and 109.00 %.
The absorbed radioactivity was predominately excreted via urine, accounting for 73.80 % (male) and 80.46 % (female) for low-dosed animals, and 64.76 % (male) and 65.73 % (female) for the high-dosed ones. Lower amounts were excreted with the faeces, accounting for 27.71 % (male) and 16.09 % (female) in the low-dosed rats, rand for 39.49 % (male) and 41.88 % (female) in the high-dosed ones.
The amount absorbed was calculated based on the radioactivity quantified in urine, cage wash, expired-air (high dose only), and the residues in carcass and tissues. At least 76.87 % (male) and 85.13 % (female) of the low dose, and 66.36 % (male) and 67.12 % (female) of the high dose, were absorbed from the gastrointestinal tract into systemic circulation.

Table B.6.1-8: Mass balance of beta-cyfluthrin derived radioactivity in rats at 0.5 and 10 mg/kg bw

		Mass balance [% of dose]			
		Group 1		Group 2	
Sex		Males	Females	Males	Females
Dose [mg/kg]		0.51	0.51	9.97	10.14
Urine					
	0-6 h	14.73	28.56	8.34	9.19
	6-24 h	38.30	38.70	39.61	46.41
	24-48 h	14.65	10.12	14.09	8.44
	48-72 h	3.17	1.25	1.82	0.86
	72-96 h	1.32	0.76	0.38	0.34
	96-120 h	0.78	0.40	0.27	0.17

	120-144 h	0.54	0.37	0.16	0.22
	144-168 h	0.30	0.30	0.10	0.12
	Subtotal	73.80	80.46	64.76	65.73
Faeces					
	0-24 h	19.18	4.39	30.94	19.21
	24-48 h	6.55	10.06	6.85	21.24
	48-72 h	1.20	1.21	0.66	0.82
	72-96 h	0.38	0.14	0.28	0.30
	96-120 h	0.16	0.08	0.23	0.07
	120-144 h	0.10	0.09	0.42	0.16
	144-168 h	0.08	0.07	0.08	0.06
	GI-Content	0.07	0.04	0.03	0.02
	Subtotal	27.71	16.09	39.49	41.88
Expired air					
	0-24 h	NA	NA	0.19	0.13
	24-48 h	NA	NA	0.09	0.07
	Subtotal	NA	NA	0.29	0.20
Cage Wash					
		2.53	4.16	0.66	0.61
Total Excretion					
	0-168 h	104.03	100.71	105.19	108.42
Tissue Residues					
	Tissues	0.13	0.10	0.11	0.08
	Carcass	0.41	0.41	0.55	0.50
	Subtotal	0.54	0.51	0.65	0.58
Amount absorbed*		76.87	85.13	66.36	67.12
Total Recovery		104.57	101.22	105.85	109.00

* The amount absorbed was calculated based on the radioactivity recovered in urine, cage wash, expired air and residues in carcass and tissues.

Blood kinetics:
The maximum concentration of radioactivity in blood was found at sampling time 0.5 hours and 6-8 hours for the low and high dose, respectively. A C_{max} of 0.1 and 0.3 µg eq/g was reached at the low dose and of 1.5 and 1.4 µg eq/g was reached at the high dose for males and females, respectively. Terminal half-life was calculated to be approximately 9-14 hours for the low dose and 42-50 hours for the high dose. AUC_{0-t} in blood was calculated to be 1.9 and 2.0 µg·h/g at the low dose and 35 and 23 µg·h/g at the high dose for males and females, respectively.

Distribution:
Mean concentrations of total radioactivity in tissues and organs are presented in the next table. Residual radioactivity 168 hours post-dose was mainly found in white fat at concentrations of approx. 0.02 µg eq/g at the low dose and of 0.4-0.5 µg eq/g at the high dose. Comparatively, concentrations in blood were below the LOQ (<0.001 µg eq/g) at the low dose and 0.006-0.008 µg eq/g at the high dose. Residual radioactivity was also found in the adrenals, gastro-intestinal tract (without content), kidneys, liver, lungs, pancreas, thyroids and ovaries (female rats).

Table B.6.1-9: Concentration of residual radioactivity in tissues and organs 168 hours post-dose

Sex	Male			Female			Male			Female		
	Mean	SD	LOQ[1]	Mean	SD	LOQ[1]	Mean	SD	LOQ[1]	Mean	SD	LOQ[1]
Dose [mg/kg bw]	0.51	0.00		0.51	0.00		10.0	0.2		10.1	0.1	
[µg equivalents per g]												
Adrenals	0.0033	0.0004	0.0022	0.0037	0.0008	0.0022	0.0757	0.0263	0.0045	0.0596	0.0213	0.0045
Blood	<LOQ	0.0001	0.0011	<LOQ	0.0001	0.0011	0.0082	0.0011	0.0045	0.0061	0.0007	0.0056
Brain	<LOQ	0.0001	0.0010	<LOQ	0.0001	0.0010	0.0041	0.0006	0.0021	0.0030	0.0004	0.0021
Fat (white)	0.0159	0.0049	0.0022	0.0201	0.0093	0.0022	0.4100	0.1585	0.0045	0.5184	0.2019	0.0045
Femur	0.0004	0.0001	0.0004	<LOQ	0.0001	0.0004	0.0097	0.0027	0.0013	0.0061	0.0020	0.0008
GI-Tract	0.0031	0.0009	0.0012	0.0041	0.0019	0.0012	0.0422	0.0101	0.0024	0.0509	0.0306	0.0024
Heart	<LOQ	0.0001	0.0011	<LOQ	0.0001	0.0011	0.0077	0.0015	0.0022	0.0062	0.0009	0.0022
Kidneys	0.0030	0.0004	0.0011	0.0040	0.0003	0.0011	0.0358	0.0070	0.0022	0.0379	0.0044	0.0022
Liver	0.0073	0.0020	0.0012	0.0040	0.0006	0.0012	0.1168	0.0294	0.0025	0.0617	0.0111	0.0025
Lungs	<LOQ	0.0001	0.0012	0.0013	0.0001	0.0012	0.0208	0.0049	0.0024	0.0200	0.0021	0.0024
Muscle	<LOQ	0.0001	0.0011	<LOQ	0.0001	0.0011	0.0063	0.0014	0.0022	0.0047	0.0011	0.0022
Ovaries	–	–	–	0.0044	0.0018	0.0022	–	–	–	0.0630	0.0177	0.0045
Pancreas	0.0018	0.0005	0.0011	0.0014	0.0005	0.0011	0.0291	0.0196	0.0022	0.0390	0.0312	0.0022
Plasma	<LOQ	0.0001	0.0011	<LOQ	0.0001	0.0011	0.0079	0.0018	0.0022	0.0062	0.0013	0.0022
Spleen	<LOQ	0.0001	0.0011	<LOQ	0.0001	0.0011	0.0080	0.0016	0.0022	0.0086	0.0025	0.0022
Testis	<LOQ	0.0001	0.0010	–	–	–	0.0052	0.0005	0.0021	–	–	–
Thymus	<LOQ	0.0003	0.0010	<LOQ	0.0002	0.0010	0.0126	0.0069	0.0021	0.0090	0.0036	0.0021
Thyroids	<LOQ	0.0017	0.0104	<LOQ	0.0037	0.0104	0.0349	0.0056	0.0211	0.0335	0.0136	0.0211
Uterus	–	–	–	0.0011	0.0009	0.0010	–	–	–	0.0252	0.0142	0.0021
Carcass	0.0022	0.0003	0.0002	0.0025	0.0004	0.0002	0.0628	0.0118	0.0005	0.0592	0.0191	0.0005

[1] LOQ: Limit of quantitation

Metabolite pattern:
Urinary metabolite pattern
The quantitative distribution of the urinary metabolite fractions is summarised in the next table (expressed as percentage of the dose).

Table B.6.1-10: Urinary metabolite pattern (sampling time 0-72 h)

	Urinary metabolite pattern [% of dose]				
Metabolite fraction	**Group 1 0.5 mg/kg bw**		**Group 2 10 mg/kg bw**		**Assignment**
	Male	Female	Male	Female	
U1	0.9	0.4	0.9	0.8	
U2	0.6	0.5	0.5	0.6	
U3	3.0	2.4	2.2	2.3	

U4	39.1	26.3	28.0	34.7	DCVA acyl glucuronide
U5	26.7	48.8	30.6	25.7	*cis/trans* DCVA
U6					
Σ	70.2	78.4	62.2	64.0	

Faecal metabolite pattern
The quantitative distribution of the faecal metabolite fractions is summarised in the next table (expressed as percentage of the dose).

Table B.6.1-11: Faecal metabolite pattern (sampling time 0-72 h)

	Faecal metabolite pattern [% of dose]				
Metabolite fraction	**Group 1 0.5 mg/kg bw**		**Group 2 10 mg/kg bw**		**Assignment**
	Male	Female	Male	Female	
F1*	5.3	1.7	3.3	1.6	
F2	8.4	4.7	7.9	4.6	*cis/trans* DCVA
F3					
F4	0.6	0.4	1.5	0.5	
F5	1.7	1.0	1.9	1.8	
F6	0.7	0.5	0.7	0.9	
F7	7.6	3.7	14.9	26.5	beta-cyfluthrin
F8	0.5	0.5	0.1	0.4	
F9	0.4	0.1	0.1	0.2	
Subtotal Non-extractable	25.1 1.9	12.8 1.5	30.4 1.8	36.6 1.4	
% dose recovered in matrix	27.0	15.7	38.4	41.3	

*F1 was taken as a cluster of peaks between the 20th and 23rd minute

Conclusion:

At both doses, male and female rats had very similar absorption, distribution, excretion and metabolism of beta-cyfluthrin.
Oral absorption from the gastrointestinal tract into systemic circulation for the low dose (0.5 mg/kg bw) accounted for at least 76.87 % and 85.13 %, and for the high dose (10 mg/kg bw) for at least 66.36 % and 67.12 % for males and females, respectively (calculated based on the radioactivity determined in urine, cage wash (residues of dried urine), expired-air (high dose only) and residues in carcass and tissues).
Information about radioactivity present in bile was not provided. Therefore, it cannot be assumed that the proportion excreted via faeces represents material which had undergone systemic absorption. A minimum oral absorption of 66 % can be assumed.
Beta-cyfluthrin was rapidly (within 48 hours after administration) excreted, predominantly via urine.
Residual radioactivity was mainly found in white fat at concentrations of 0.02 µg beta-cyfluthrin eq/g at the low dose and of 0.4-0.5 µg beta-Cyfluthrin eq/g at the high dose.
The investigation of the metabolite pattern in urine and faeces revealed that beta-cyfluthrin was extensively metabolised for both doses and sexes. Urinary metabolite pattern consisted of at least 6 metabolite fractions. The major metabolites were fractions U4, U5 and U6 (glucuronide conjugate of DCVA and *cis/trans* DCVA). All other fractions were ≤3 % of dose. No unchanged parent was detected in urine whereas it was the major test-related material found in faeces. The faecal metabolite pattern re-

vealed at least 9 metabolite fractions. The metabolite pattern was dominated by three major fractions, i.e. F2 and F3 (3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA)) and F7 (beta-Cyfluthrin).
The proposed metabolic pathway is the following: beta-cyfluthrin → DCVA → DCVA glucuronide conjugate.

The study is considered acceptable under the conditions of the study and based on the information given in the report.

Data point:	KCA 5.1.2
Report:	Hassler, 2014, ASB2014-7719 Beta-cyfluthrin: Comparative *in-vitro* Metabolism of [fluoro-phenyl-UL-^{14}C] beta-cyfluthrin in Rat and Human Liver Microsomes. Company: Irvita Plant Protection. Report No: D59934, M-482993-01-1
Guideline(s):	No guideline available
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Acceptable

(Dates of exp. work: August 2012- September 2012)

Materials and methods:

Test substance: beta-cyfluthrin, radiolabeled test item: batch number: PNBC000623, isomer distribution: I: max. 2 %, II: 30-40 %, III: max. 3 %, IV: 57.0-67 %, purity 99.3 %.
Rat liver microsomes from male Wistar rats (HAN), source: XenoTech, Kansas, USA;
Human liver microsomes from males, source: XenoTech, Kansas, USA

[Fluorophenyl-UL-^{14}C]-beta-cyfluthrin was incubated with either rat or human active microsomes, heat inactivated microsomes and in the absence of microsomes and co-factors. In order to show the suitability of the test system a standard substrate, i.e. [^{14}C] testosterone, was incubated under the same experimental conditions. Both substances were pre-diluted in methanol to generate stock solutions.
Based on the obtained results of a pre-test the final target substrate concentration was selected at 10 µM, corresponding to 4.2 µg beta-cyfluthrin/mL and 0.5 mg/mL active liver microsomes of both test species.
The following procedure was executed with both test system, i.e. rat and human liver microsomes.

Table B.6.1-12: Incubation at main test with rat and human liver microsomes

Sample no.	Substrate concentration	Incubation time	Microsome status*
[fluorophenyl-UL-^{14}C]-beta-cyfluthrin			
1	10 µM (4.2 µg/mL)	0 hours	Without
2			
3		0.25 hours	
4			
5		0.5 hours	
6			
7		1 hours	
8			

9	10 µM (4.2 µg/mL)	0.25 hours	Inactivated**
10			
11		0.5 hours	
12			
13		1 hours	
14			
15	10 µM (4.2 µg/mL)	0.25 hours	Active
16			
17		0.5 hours	
18			
19		1 hours	
20			
[^{14}C] testosterone			
21	10 µM (2.8 µg/mL)	0.25 hours	Without
22		1 hours	
23	10 µM (2.8 µg/mL)	0.25 hours	Inactivated**
24		1 hours	
25	10 µM (2.8 µg/mL)	0.25 hours	Active
26		1 hours	

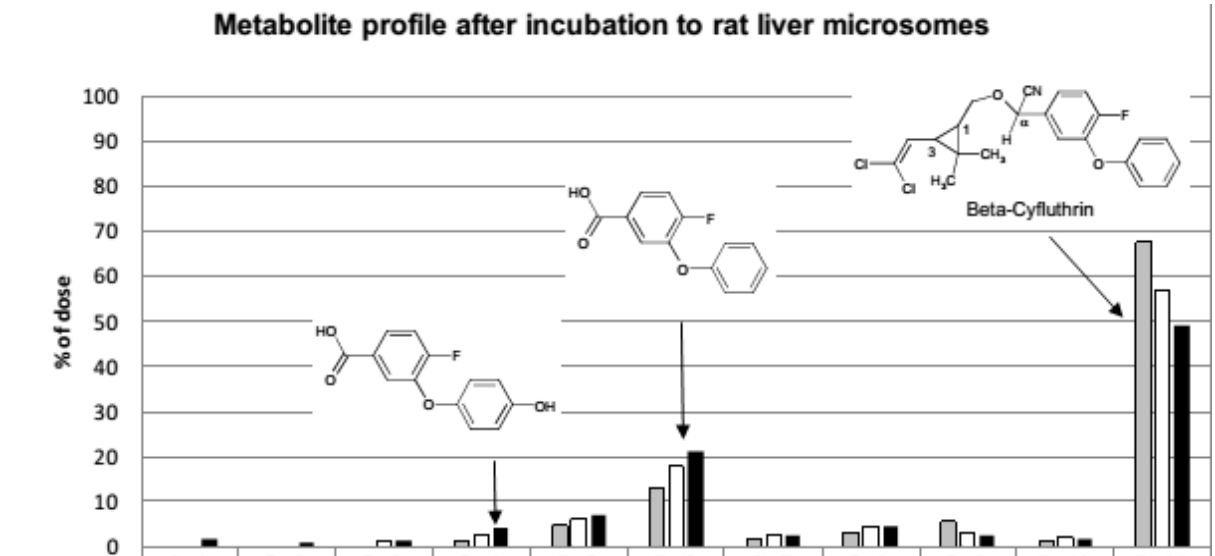
* 0.5 mg/mL microsome concentration (where applicable)
** heated for 10 min at 80 °C

At the end of the respective incubation time 1000 µL cooled (about 0 °C) methanol:water HCl 1M (8:2, v/v) was added to the respective flask, inducing protein precipitation. Thereafter the supernatant was separated by centrifugation (3000 rpm/10 min/RT).The proteins were extracted two times with 1 mL methanol each. The remaining proteins were dissolved to determine remaining radioactivity in the pellet.

The metabolite patterns of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin were investigated in the pooled supernatants of the samples. Radioactivity in the total volume of the supernatant and the precipitate was determined by Liquid Scintillation Counting (LSC) in each sample. Aliquots of the supernatant were analysed by HPLC.

Results and discussions:

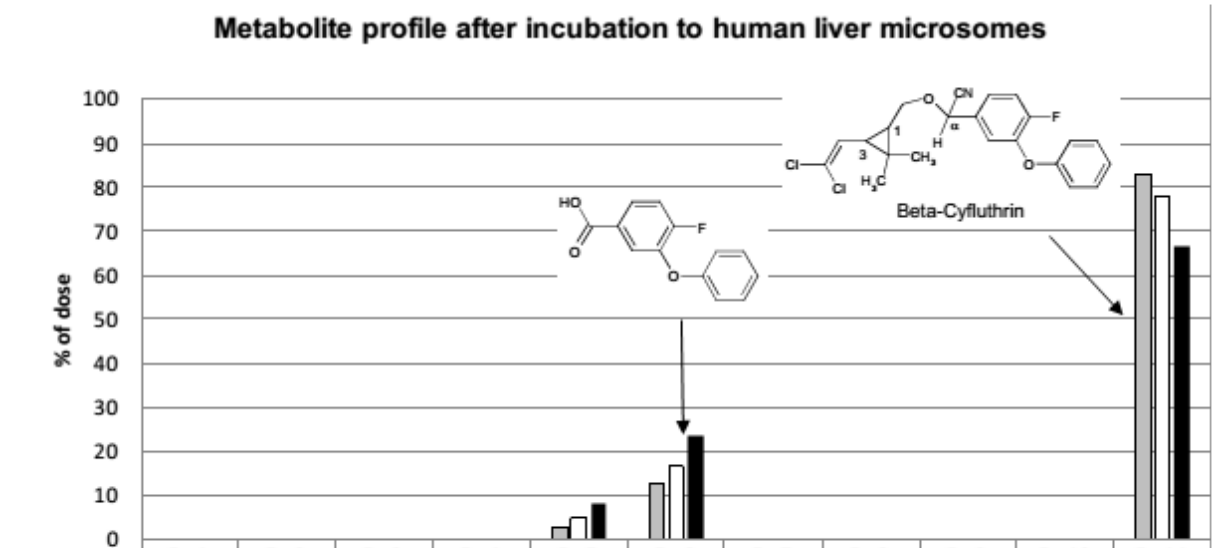
Metabolite pattern:
After incubation of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin with active rat liver microsomes in the presence of NADPH regeneration system the test item was extensively metabolised. The *in vitro* metabolite pattern (see Figure B.6.1-1) consisted of at least 11 metabolite fractions. With increasing incubation time the qualitative metabolite pattern did change slightly and the quantitative values showed a decrease of the parent compound and consequently an increase of the metabolite fractions. Within 1 hour of incubation the amount of parent decreased to 49.3 % of the dose, whereas the major metabolite fractions (Fr. 6, 5, 8, 4) increased to 21.1 %, 7.1 %, 4.7 %, and 4.0 % of the dose, respectively. Two of the metabolite fractions could be assigned by co-chromatography to reference items, i.e. Fr. 6 = FPB-acid (4-fluoro-3-phenoxybenzoic acid), Fr. 4 = OH-FPB acid (3(4'-hydroxyphenoxy)-4-fluorobenzoic acid).



	Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5	Fr. 6	Fr. 7	Fr. 8	Fr. 9	Fr. 10	Fr. 11
0.25 hours	0.0	0.0	0.0	1.0	4.8	13.0	1.7	3.2	5.5	1.3	67.7
0.5 hours	0.0	0.0	1.2	2.6	5.8	17.9	2.7	4.5	3.3	2.1	57.2
1 hour	1.6	0.9	1.6	4.0	7.1	21.1	2.6	4.7	2.7	1.6	49.3

Figure B.6.1-1: Metabolite profile of [^{14}C] beta-cyfluthrin after incubation to rat liver microsomes

After incubation of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin with active human liver microsomes the metabolism rate was limited. The metabolite pattern (see Figure B.6.1-2) consisted of two metabolite fractions. The qualitative metabolite pattern did not change with ongoing incubation time, but the quantitative values showed a decrease of the parent compound and consequently an increase of the metabolite fractions. Within 1 hour of incubation the amount of parent decreased to 66.4 % of the dose, whereas the metabolite fractions Fr. 6 and 5 increased to 23.8 %, and 8.1 % of the dose, respectively.



	Fr. 1	Fr. 2	Fr. 3	Fr. 4	Fr. 5	Fr. 6	Fr. 7	Fr. 8	Fr. 9	Fr. 10	Fr. 11
0.25 hours	0.0	0.0	0.0	0.0	3.0	13.0	0.0	0.0	0.0	0.0	83.0
0.5 hours	0.0	0.0	0.0	0.0	4.6	16.4	0.0	0.0	0.0	0.0	77.6
1 hour	0.0	0.0	0.0	0.0	8.1	23.8	0.0	0.0	0.0	0.0	66.4

Figure B.6.1-2: Metabolite profile of [^{14}C] beta-cyfluthrin after incubation to human liver microsomes

Conclusion:

After incubation of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin with active rat liver microsomes in the presence of NADPH regeneration system the test item was extensively metabolised. The *in-vitro* metabolite pattern consisted of at least 11 metabolite fractions.
The metabolism after incubation with active human liver microsomes was limited compared to rat liver microsomes. The metabolite pattern consisted just of two metabolite fractions. All metabolite fractions observed in human microsomes were also found in rats in similar portions.
The study is considered acceptable under the conditions of the study and based on the information given in the report.

Literature research for the Renewal Assessment Report (RAR):

Data point:	KIIA 5.10
Report:	Scollon et al. (2009) ASB2015-931 *In vitro* Metabolism of Pyrethroid Pesticides by Rat and Human Hepatic Microsomes and Cytochrome P450 Isoforms. Drug metabolism and disposition Vol. 37, No. 1 DMD 37:221–228, 2009
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
Species differences in the intrinsic clearance and the enzymes involved in the metabolism of pyrethroid pesticides were examined in rat and human hepatic microsomes. Different pyrethroids including beta-cyfluthrin were incubated in rat and human hepatic microsomes in the presence or absence of NADPH. Metabolism was measured using a parent depletion approach. The intrinsic clearance of the majority of pyrethroids was 5 to 15-fold greater in rat relative to human microsomes. The metabolism

of beta-cyfluthrin in microsomes from both species was metabolised by both oxidative and hydrolytic pathways. Rat cytochrome P450 isoforms that showed activity toward several pyrethroids included CYP1A1, CYP1A2, CYP2C6, CYP2C11, CYP3A1, and CYP3A2. Human P450 isoforms that showed activity toward multiple pyrethroids were CYP2C8, CYP2C9, CYP2C19, and CYP3A4. Species-specific differences in metabolism may result in variable detoxification of pyrethroids, which may in turn result in divergent neurotoxic outcomes. These species differences and isomer interactions in metabolism of pyrethroids should be considered when assessing the potential adverse health effects of pyrethroid pesticides.

Conclusion:

This publication supports the results in the study of Hassler (2014, ASB2014-7719), who showed that after incubation of [fluorophenyl-UL-^{14}C]-beta-cyfluthrin with active rat liver microsomes in the presence of NADPH regeneration system the test item was extensively metabolised.

The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Anadón et al. (2013) ASB2015-926 Differential induction of cytochrome P450 isoforms and peroxisomal proliferation by cyfluthrin in male Wistar rats. Toxicology Letters 220 (2013) 135-142
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
Cyfluthrin effects on *in vivo* drug metabolising enzymes were evaluated using the oxidative substrate antipyrine. Antipyrine pharmacokinetics in plasma and urinary excretion of its major metabolites with and without cyfluthrin oral treatment (20 mg/kg/day for 6 days) were investigated in rats. Cyfluthrin increased the apparent intrinsic clearance and decreased the antipyrine half-life at beta phase. Cyfluthrin also increased the clearance of the antipyrine metabolites, norantipyrine, 4-hydroxyantipyrine and 3-hydroxymethylantipyrine and the formation rate constants for each of the three metabolites measured in urine. These results suggest that cyfluthrin affects hepatic cytochrome P450 (CYP) system. In order to confirm, a second experiment was carried out. The authors evaluated the effects of repeated exposure to cyfluthrin on hepatic and renal CYP2E, CYP1A and CYP4A subfamilies and peroxisomal proliferation in rats following oral administration (10 and 20 mg/kg/day for 6 days). At the highest dose, cyfluthrin increased renal and hepatic O-deethylation of ethoxyresorufin and O-demethylation of methoxyresorufin, metabolism mediated by the CYP1A subfamily. Liver and kidney were susceptible to cyfluthrin-dependent induction of 12- and 11-hydroxylation of lauric acid, suggesting CYP4A subfamily induction. Also cyfluthrin increased the beta-oxidation of palmitoyl-coenzyme A and carnitine acetyltransferase activity, supporting cyfluthrin as a peroxisome proliferator.

Conclusion:

The results suggest that cyfluthrin induced hepatic CYP1A, CYP4A subfamilies and acts as a peroxisome proliferator. The study results are considered to represent supplemental information.

B.6.1.2 Absorption, distribution, metabolism and excretion by other routes

Two new dermal penetration study with the representative products, Bulldock 25EC (Maas, 2013, ASB2014-7885) and Montur Forte FS 230 (Odin, 2014, ASB2014-7895), has been performed (see Volume 3 CP B.6 Bulldock EC25 and Volume 3 CP B.6 Montur Forte FS 230). Previously, a default value of 10 % dermal absorption is used for risk assessment, based on the physico-chemical properties of pyrethroids in general.
Based on these new studies the dermal penetration estimates to be used for risk assessment were set at 13 % and 37 % for the formulation concentrate and 1:2000 field spray dilutions, respectively (Bulldock EC 25) and 1 % for the concentrate (80 g/L); 0.3 % for the intermediate dose (40 g/L), and 0.7 % for the low dose (11.4 g/L) (Montur Forte FS 230).

B.6.1.3 Further studies

The following studies in farm animals were included in the human health section of the original monograph of beta-cyfluthrin (October 1996; ASB2010-10436). However, they are considered not relevant for the human health evaluation. Therefore, these studies are not assessed in the Renewal Assessment Report (RAR, 2015).

Original monograph of beta-cyfluthrin by the RMS in October 1996 (ASB2010-10436):

Report: [redacted] 1983, RIP9400870:
Metabolism of Baythroid™ in a dairy cow. Report No.: MR 86043 (September 27, 1983, 1st revised October 15, 1984, 2nd revised August 5, 1985); [redacted]

Report: [redacted]., 1983, RIP9400869:
The distribution and metabolism of Baythroid™ in laying hens. Report No.: MR 86044, (September 20, 1983); [redacted]

Report: [redacted]., 1987, TOX9401851:
Biotransformation of cyfluthrin in the chicken after oral administration of a high dose. Report No.: PH 15849, (June 24, 1987); [redacted]

B.6.2 Acute toxicity

The following new studies with the active substance have been conducted on acute endpoints after Annex I inclusion. The first six have been conducted to address a national data requirement of Brazil. In addition, to meet the new requirements under Regulation 1107/2009 a phototoxicity study was performed.

Acute oral toxicity in the rat ([redacted], 2005, ASB2014-7720)
Acute percutaneous toxicity in the rat ([redacted], 2005, ASB2014-7721)
In vitro corrosivity assay on reconstructed human epidermis [redacted], 2005, ASB2014-7722)
In vivo skin irritation study in rabbits [redacted], 2005, ASB2014-7723)
In vivo eye irritation study in rabbits [redacted], 2005, ASB2014-7724)
Skin sensitisation test (Buehler method) [redacted], 2005, ASB2014-7725)
Phototoxicity ([redacted]r, 2013, ASB2014-7726)

B.6.2.1 Oral

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.1 /01
Report:	[redacted] 1987, TOX9550258 FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in polyethylene glycol E 400). Report no.: 16182 (November 5, 1987); [redacted]y
Guideline(s):	The test was run according to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987). Besides fasted also fed rats were tested (recommended in OECD TG, 1981 and 1987). The negative controls and administered volumes were not mentioned in the report (only in the dossier).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study (part with fasted animals) is acceptable.

(dates of exp. work: March - April 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered once by gavage to Wistar rats (Bor: WISW [SPFCpb], source: [redacted]) in the following dosing schedule:
0-10-100-630-800-1000-1400-2500 mg/kg bw, 5 male non-fasted rats/dosage group,
0-10-100-1000-1400-1800-2000 mg/kg bw, 5 female non-fasted rats/dosage group,
0-10-50-100-250-500-710-1000-1400 mg/kg bw, 5 fasted male rats/dosage group,
0-10-50-100-800-1000-1400-1500-1600-2000 mg/kg bw, 5 fasted female rats/dosage group.
The formulating agent was PEG 400. The administered volumes were 5 (10-1000 mg/kg bw) or

10 ml/kg bw (>1000 mg/kg bw). Fasted animals were fed 2 h after dosing.
Recording period: 0-14 days.
Bw: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-1: Oral LD_{50} in non-fasted and fasted rats

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Non-fasted male rats	PEG 400	10	655 (395-1088)
Non-fasted female rats	PEG 400	10	1369 (1137-1651)
Fasted male rats	PEG 400	10	380 (231-625)
Fasted female rats	PEG 400	10	651 (329-1294)

= maximum dosage without clinical signs.
* = () confidence interval (95 %).

Onset of death: Within hours to 7 days maximum.
Clinical signs were observed in fasted rats from a dose of 50 mg/kg bw onward. To the findings belonged increased activity and digging and preening movements (beginning at ca. 15 min for up to ca. 4 h); lethargy and salivation (beginning at ca. 30 min for up to ca. 12 days); uncoordinated gait, splayed gait, difficult breathing, occasional rolling, piloerection and soft faeces (beginning at ca. 2 h for up to ca. 11 days).
Clinical signs were observed in fed rats from a dose of 100 mg/kg bw. To the findings belonged increased activity and digging and preening movements (beginning at ca. 15 min for up to ca. 4 h); lethargy, salivation uncoordinated gait, splayed gait, difficult breathing, occasional rolling, piloerection and soft faeces (beginning at ca. 30 min for up to ca. 12 days).
No delayed effects.
Body weight: Starting at 100 mg/kg bw (non-fasted rats) or in the lethal dose range (fasted rats) a reducing effect on body weight gain was observed, but compensated at day 14.
Gross pathology: Animals dying intercurrently: Lung: distended, mottled, occasionally dark red (fasted und male fed rats) or fluid (only fasted rats) in the tissue; kidney: mottled, occasionally pale (only fed rats); spleen: mottled, occasionally pale (only fed rats); liver: mottled, occasionally slight lobular pattern; gastrointestinal tract: distended, empty or filled with fluid content (only fed rats) in some cases; glandular stomach: reddened (fasted males only), several ulcer-like foci in the glandular stomach (fasted rats only).
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

The duration of clinical signs and the period when death occurred provided evidence of a prolonged effect of the test compound. There was a complete reversibility of toxic action observed at the end of observation period. Overall the fasted rats were more sensitive than the non-fasted rats. The males showed a greater sensitivity than the females.
Beta-cyfluthrin was of moderate acute toxicity to rats after oral administration in PEG 400.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the result and discussion section were revised.
This acute oral toxicity study was based on OECD-Guideline no. 401 (adopted May 12, 1981).

OECD-Guideline no. 401 advised the utilisation of fasted animals for LD_{50} identification. However, in this study both fed and fasted rats were used for LD_{50} determination. Furthermore, for fasted animals food should be withheld for 3-4 h after substance administration instead of 2 h.
Under the conditions of the study and based on the information given in the report, LD_{50} values (test compound formulated in polyethylene glycol E 400) for fasted male and female rats were 380 mg/kg bw and 651 mg/kg bw, respectively. Fed male and female rats were less susceptible reflected by higher LD_{50} values (655 and 1369 mg/kg bw, respectively).
No clinical signs were observed in fed and fasted rats of both sexes at the low dose of 10 mg/kg bw. In contrast to this, all other doses were connected with clinical signs. For example, splayed and uncoordinated gait occurred in fasted rats from a concentration of 50 mg/kg bw and in fed rats from a concentration of 100 mg/kg bw.

Data point:	KCA 5.2.1 /03
Report:	[redacted] 1987, TOX9550257 FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in acetone/peanut oil). Report no.: 16181 (November 5, 1987); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987). Besides fasted also fed rats were used (recommended in OECD TG, 1981 and 1987). The negative controls were not mentioned in the report (only in the dossier).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study (part with fasted animals) is acceptable.

(dates of exp. work: March - April 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered once by gavage to Wistar rats (strain Bor:WISW [SPFCpb], source: [redacted]) in the following dosing schedule:
0-1-10-100-160-180-200 mg/kg bw, 5 non-fasted male rats/dosage group,
0-1-10-71*-100-160-200-250 mg/kg bw, 5 non-fasted female rats/dosage group (* 10 animals),
0-1-10-71-100-160-250 mg/kg bw, 5 fasted male rats/dosage group,
0-1-10-63-80-100-160 mg/kg bw, 5 fasted female rats/dosage group.
The formulating agent was acetone/peanut oil (1/10), the administered volume 5 ml/kg bw. Fasted animals were fed 2 h after dosing.
Recording period: 0-14 days.
Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-2: Oral LD_{50} in non-fasted and fasted rats

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Non-fasted male rats	acetone/ peanut oil	1	141 (113-177)
Non-fasted female rats	acetone/ peanut oil	1	108 (78-152)
Fasted male rats	acetone/ peanut oil	1	84 (55-131)
Fasted female rats	acetone/ peanut oil	1	77 (65-93)

= maximum dosage without clinical signs.
* = () confidence interval (95 %).

Onset of death: Within hours to 3 days maximum.
Clinical signs (10 mg/kg bw-group): Lethargy, cramped posture as well as digging and preening movements (non-fasted animals only) (ca. 1 h to 3 days).
Other groups: lethargy, uncoordinated gait, digging and preening movements, cramped posture, splayed gait, rolling, salivation, laboured/difficult breathing and piloerection, increased activity (only fed rats) , soft faeces (only fed rats) (ca. 40 min for up to a maximum of 10 days).
No delayed effects.
Body weight - Fasted rats: Starting at 71 mg/kg bw (males) or 80 mg/kg bw (females): impact on pattern of weight gain, later compensated.
Body weight - Fed rats: occasional males starting at 100 mg/kg bw and occasional females starting at 10 mg/kg bw with weight loss, later compensated.
Gross pathology: Animals dying intercurrently: Lung: mottled to dark red, slightly distended; kidney: occasionally mottled (only fasted rats and fed female rats), slightly marbled (only fasted rats) and/or pale (only male fed rats); spleen: occasionally mottled (only fasted rats and fed female rats) and/or pale (only male fed rats); liver: mottled, occasionally pale with a lobular pattern (only fed and fasted female rats); gastrointestinal tract: in some cases distended, filled with mucus (and shavings) in some cases (only fasted rats), detachment of the mucosa in the fore stomach in some cases (only fasted and fed female rats).
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

Beta-cyfluthrin was of high acute toxicity to fasted and non-fasted rats after oral administration in acetone/peanut oil. The fasted animals were somewhat more susceptible.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the result and discussion section were revised.
The acute oral toxicity study presented complied with OECD-guideline no. 401 (adopted May 12, 1981). The applied OECD-Guideline 401 requires the use of fasted animals for oral LD_{50} determination. Nevertheless, the submitted study operated with fasted and fed rats. Furthermore, for fasted animals food should be withheld for 3-4 h after substance administration instead of 2 h.
Under the conditions of the study and based on the information given in the report, oral LD_{50} values (test compound formulated in acetone/peanut oil) for fed male and female rats were 141 and 108 mg/kg bw, respectively. The use of fasted male and female rats resulted in lower LD_{50} values (84 and 77 mg/kg bw) alluding to higher susceptibility.

The lowest dose (1 mg/kg bw) was tolerated in fasted and fed rats of both sexes without exhibiting clinical signs. However, from a concentration of 10 mg/kg bw clinical signs (e.g. lethargy) were observed in fasted and fed rats.

Data point:	KCA 5.2.1 /02
Report:	[redacted]. 1987, TOX9550255 FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in xylene). Report no.: 16176 (November 4, 1987); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987). Besides fasted also fed rats were tested (recommended in OECD TG, 1981 and 1987).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study (part with fasted animals) is acceptable.

(dates of exp. work: March - April 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered once by gavage to Wistar rats (strain Bor:WISW [SPFCpb], source: [redacted]) in the following dosing schedule:
0-1-10-100-200-250-315-355-400-500 mg/kg bw, 5 male non-fasted rats/dosage group,
0-1-10-100-250-355-400-450-500 mg/kg bw, 5 female non-fasted rats/dosage group,
0-1-10-50-100-250-400-500 mg/kg bw, 5 fasted male rats/dosage group,
0-1-10-100-250-315-400-500 mg/kg bw, 5 fasted female rats/dosage group.
The formulating agent was xylene, the administered volume 1 ml/kg bw. Fasted animals were fed 2 h after dosing.
Recording period: 0-14 days.
Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-3: Oral LD_{50} in non-fasted and fasted rats

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Non-fasted male rats	xylene	1	307 (260-364)
Non-fasted female rats	xylene	1	343 (286-411)
Fasted male rats	xylene	1	211 (110-404)
Fasted female rats	xylene	1	336 (290-391)

= maximum dosage without clinical signs which are related to the active substance.
* = () confidence interval (95 %).

Onset of death: Within hours to 3 days maximum.

Clinical signs: Control group and 1 mg/kg bw-group: Lethargy, reduced activity, difficult breathing (ca. 40 min to 1 day).
Other groups: Lethargy, uncoordinated gait, digging and preening movements, cramped posture, splayed gait, rolling, salivation, difficult breathing and piloerection (beginning at ca. 30 min for up to a maximum of 9 days). No delayed effects.
Body weight fasted rats: On the whole no effect (occasionally slight weight loss in males starting at 50 mg/kg bw and in females starting at 100 mg/kg, later compensated)
Body weight fed rats: On the whole no effect (bw loss in males starting at 100 mg/kg bw and in females starting at 10 mg/kg, later compensated)
Gross pathology: Animals dying intercurrently: Lung: mottled to dark red, slightly distended; kidney: mottled, occasionally slightly marbled (only fasted rats); spleen: mottled, somewhat pale in some cases (only fed rats); liver: mottled, occasionally pale and/or a lobular pattern (only fasted female and fed rats), occasionally pale and beige discoloration (only fed rats); gastrointestinal tract: distended in some cases, partially empty; occasionally with dark mucous content (only fed rats), detachment of the mucosa in the fore stomach, occasionally ulcer-like foci in the glandular stomach (fasted males only).
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion

Beta-cyfluthrin was of moderate acute toxicity to fasted and non-fasted rats after oral administration in xylene.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the result and discussion section were revised.
Under the conditions of OECD-guideline no. 401 (adopted in May 12, 1981) for acute oral toxicity, the study was conducted. Although in accordance with this guideline only fasted animals should be taken for oral LD_{50} determination, the provided study supplied LD_{50} values for fasted and fed rats as well. Furthermore, for fasted animals food should be withheld for 3-4 h after substance administration instead of 2 h.
Under the conditions of the study and based on the information given in the report, oral LD_{50} values (test compound formulated in xylene) for fed male and female rats amounted to 307 and 343 mg/kg bw, respectively. Fasted male and female animals were more susceptible represented by lower LD_{50} values (211 and 336 mg/kg bw).
Test material-related clinical signs (e.g. uncoordinated gait and cramped posture) occurred in fasted and fed animals from a dose of 10 mg/kg bw.

Data point:	KCA 5.8.2 /03
Report:	[redacted] 1982, TOX9401854 FCR 1272 - Comparative tests with various formulation aids – Report no.: 10931 (June 07, 1982); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B1.
Deviations:	Necropsy was not performed (recommended in OECD TG, 1981 and 1987). The food duration for fasting after dosing was not mentioned in the report (only in the dossier). Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987).

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is acceptable.

(Dates of exp. work: July 1981 to December 1981).

Materials and methods:

Groups of 5-20 fasted male albino rats (strain WISW [SPF, CPB], source: [redacted]) received cyfluthrin (batch no: 816170019, purity: 95.0 %) via single administration in the following dosing schedule:
13-15-17.5-20 mg/kg bw in cremophor EL/water;
200-250-300-350-500 mg/kg bw in acetone/oil;
125-150-200-350-500-750-1000 mg/kg bw in dimethyl sulfoxide or
100-250-500-1000 mg/kg bw in *N*-methylpyrrolidone.
Food was provided 2 h after dosing.
Recording period: 0-14 d; bw not determined, necropsy not performed.
Statistical method: Litchfield and Wilcoxon's test.

Results and discussions:

Table B.6.2-4: LD_{50} in rats in dependence of the formulating agent

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Fasted male rats	Cremophor EL/water	<13	16.2 (13.5-19.5)
Fasted male rats	acetone/oil	<200	254 (220-294)
Fasted male rats	dimethyl sulfoxide	<125	396 (317-494)
Fasted male rats	*N*-methylpyrrolidone	<100	500-1000

= maximum dosage without clinical signs.
* = () is (most probably) confidence interval (95 %).

Clinical signs: The signs indicate an effect on the central nervous system (tremor, rolling movements, disturbed motility and respiration). Onset of symptoms arose within 1 h and was apparent for 1 to 5 days.

Conclusion:

The acute oral toxicity of cyfluthrin depends on the formulating agent on a high degree. By reason of the high toxicity in cremophor EL/water the classification "very toxic" is proposed.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the result and discussion section were revised.
A guideline for the tests regarding oral LD_{50} determination was not mentioned in the report. However, the study is similar to OECD-Guideline no. 401. Acute oral toxicity of the test compound considering various formulation aids was investigated experimentally using unfed male rats.
Under the conditions of the study and based on the information given in the report, the test result turned out to be strongly dependent on the formulation aid. Lowest LD_{50} was sought after application of the test compound in cremophor EL/distilled water (16.2 mg/kg bw), followed by acetone/oil (254 mg/kg bw), dimethyl sulfoxide (396 mg/kg bw) and *N*-methylpyrrolidine (500-1000 mg/kg bw).
Clinical signs were already observed at the lowest concentration (cremophor EL/distilled water: 13 mg/kg bw; acetone and oil: 200 mg/kg bw; dimethyl sulfoxide: 125 mg/kg bw; *N*-methylpyrrolidone: 100 mg/kg bw). The symptoms (tremor, rolling movements, disturbed motility and respiration) indicate an impact on the central nervous system. However, from the report it remains unclear, which symptom can be allocated to which dose level or solvent. Furthermore, necropsy was not performed.

Data point:	KCA 5.2.1 /06
Report:	[redacted] 1987, TOX9550256 FCR 4545 technical - Study of the acute oral toxicity to mice (formulation in polyethylene glycol E 400). Report no.: 16177 (November 4, 1987); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987). The negative controls were not mentioned in the report (only in the dossier).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: March - April 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered once by gavage to mice of the strain Bor:WISW (SPF-Han), source: [redacted]) in the following dosing schedule:
0-10-25-50-71-100-160-250-500 mg/kg bw, 5 fasted male mice/dosage group,
0-10-50-71-100*-160-200-224-250-500 mg/kg bw, 5 fasted female mice/dosage group (*10 animals).
The formulating agent was PEG 400, the administered volume 5 mL/kg bw. Food was provided 2 h after dosing.
Recording period: 0-14 days.
Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-5: Oral LD_{50} in fasted mice

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Fasted male mice	PEG 400	10	91 (58-146)
Fasted female mice	PEG 400	10	165 (137-200)

= maximum dosage without clinical signs.
* = () Confidence interval (95 %).

Onset of death: Within 1 h to 2 days maximum.
Clinical signs: Only low dose (10 mg/kg bw) was tolerated without showing clinical signs.
Other doses: Lethargy, increased activity, digging and preening movements, uncoordinated gait, splayed gait, rolling, salivation, difficult breathing (beginning at ca. 30 min for up to a maximum of 4 days). No delayed effects.
Body weight: No effect.
Gross pathology: Animals dying intercurrently: Lung: distended; kidney: mottled, slightly marbled (females only), occasionally pale; liver: pale, with a lobular pattern, occasionally mottled (females only); stomach: occasionally distended, empty or filled with mucus in some cases; small intestine:

filled with mucus.
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

The maximum duration of signs of 2 to 4 days provided evidence of a relatively rapid reversibility of the toxic effects in mice. The females were less sensitive than the males.
Beta-cyfluthrin was of high acute toxicity to mice after oral administration in PEG 400.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the result and discussion section were revised.
This oral acute toxicity study was run according to OECD-guideline no. 401 (adopted in May 12, 1981).
The study was conducted with fasted male and female mice. The test compound was formulated in polyethylene glycol E 400. Under the conditions of the study and based on the information given in the report, male mice turned out to be more susceptible (LD_{50} 91 mg/kg bw) than female mice (LD_{50} 165 mg/kg bw). Food was withheld for 2 h instead of 3-4 h after substance administration.
The substance was tolerated in both sexes without developing clinical signs up to a concentration of 10 mg/kg bw. From the next higher dose (male: 25 mg/kg bw; female: 50 mg/kg bw) to the highest dose applied in the study clinical signs (e.g. uncoordinated and splayed gait) were observed.

Data point:	KCA 5.2.1 /07
Report:	[redacted]. 1985, TOX9550254 Range finding test for acute toxicity to the dog. Report no.: 13726 (August 14, 1985); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compulsory.
Deviations:	Not applicable. Critical points of the study: One dog per dose and sex. Vomiting of dogs changes real dose.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered not acceptable.

(dates of exp. work: April - July, 1985).

Materials and methods:

One male and one female beagle dog per dose (strain BOR:BEAG, source [redacted]) received beta-cyfluthrin (batch no.:16002/84, purity: 98.5 %) orally in doses of 2500 and 5000 mg/kg bw. The substance was applied in 0.5 % aqueous tylose in a volume of 12.5 and 25 ml/kg bw, respectively. The application was performed by gavage in the morning before feeding (fasted state).
Recording period: 0-14 days.
Body weight: Day 0, 7, 14.
Necropsy: not performed.

Results and discussions:

Oral LD_{50} >5000 mg/kg bw.
Clinical signs: Vomiting (for up to 5 h), reduced appetite for up to 3 days post-treatment (females only).

Conclusion:

A single oral administration of up to 5000 mg/kg bw beta-cyfluthrin did not result in marked systemic toxic signs. However the real beta-cyfluthrin intake is not known, due to the emetic effect of the test substance on dogs. Thus the LD_{50} >5000 mg/kg bw is a questionable value.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be supplementary. However, due to the deviations listed below the study is now considered to be no longer acceptable.
The acute oral toxicity was studied in fasted beagle dogs. At the time the study was conducted, no suitable OECD-guideline was compulsory.
It is criticisable that the whole experiment was performed with 1 dog per dose group and sex. Each one male and female dog received a suspension of the test compound in 0.5 % tylose solution corresponding to 2500 mg/kg body weight or 5000 mg/kg body weight. A rationale for choosing tylose solution as vehicle was not given. The observation time after administration of the test compound covered 14 days. Under the conditions of the study and based on the information given in the report, neither lethality nor special toxic effects were noticeable. However, vomiting (both doses) soon after administration hampered the determination of the oral LD_{50} value as the exact intake of the test substance remained unclear. For this reason it is also difficult to derive a NOAEL value.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.2.1 /16
Report:	[redacted] 2005, ASB2014-7720 Beta-cyfluthrin (FCR 4545) - Acute toxicity in the rat after oral administration. Report No: AT02686 (December 8, 2005), M-263158-01-1; [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 423 (2001).
Deviations:	No rationale for the solvent was provided (demanded in OECD TG 2001).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: November 03, 2005 – November 24, 2005).

Materials and methods:

Beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %) was administered once by gavage to female Wistar rats (strain HsdCpb:Wu, source: [redacted]) in the following dosing schedule:
50 (1^{st}) – 50 (2^{nd}) – 300 mg/kg bw, 3 fasted female rats/dose.
The formulating agent was acetone/corn oil 1/10 and the administered volume amounted to 10 ml/kg bw. Food was provided 2-4 h after dosing.
At the beginning, 3 female rats were treated with 300 mg/kg bw. After 2 out 3 rats had died in the first group, two groups (each 3 females) were sequentially dosed with 50 mg/kg bw (next lower dose in the decision tree laid down in OECD TG 423). Since all animals of the 50-mg/kg groups survived till the end of the study, no further dose groups were necessary.
Observation period: 0-14 days.
Body weight: Weekly (day 1, 8/9, 15).

Necropsy: All animals (survivors sacrificed by carbon dioxide treatment).
Estimation of LD_{50} value according decision tree presented in the test guideline.

Results and discussions:

Table 6.2-6: Oral LD_{50} in fasted rats

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} cut-off [mg/kg bw]*
Fasted male rats	acetone/corn oil (1/10)	50	200

= maximum dosage without clinical signs.
* = based on dicison tree in OECD-Guideline no. 423.

Onset of death: 2 days after treatment (only 2 out of 3 animals of high dose group).
Only low dose (50 mg/kg bw) was tolerated without developing clinical signs.
Clinical signs (300 mg/kg bw): Decreased motility, uncoordinated gait, increased salivation, spasmodic state, narrowed palpebral fissure, abdominal position, temporary rolling over, and temporary creeping gait (beginning at ca. 1 h for up to a maximum of 6 days).
No delayed effects.
Body weight: No effect.
Gross pathology: No particular findings in animals treated with 50 mg/kg be and in the surviving animal treated with 300 mg/kg bw.
Animals dying during observation interval: Discoloured and spotted liver and gas-filled stomach.

Conclusion:

To investigate the acute oral toxicity of beta-cyfluthrin, OECD-Guideline no. 423 (adopted in December 17, 2001) was applied. The study was conducted with fasted female rats and is considered acceptable.
A mixture of acetone/corn oil served as formulation agent. However, a rationale for this solvent was not provided.
Under the conditions of the study and based on the information given in the report, the LD_{50} value of beta-cyfluthrin was in the range >50-300 mg/kg bw (according to the decision tree presented in OECD-Guideline no. 423).
Clinical signs (e.g. decreased motility, uncoordinated gait and spasmodic state) were only observed in animals treated with 300 mg/kg body weight of the test compound.

Further studies available to the RMS:

The studies listed below were mostly not submitted by the applicant for renewal of approval (exceptions are marked in the table). However, the studies are available to the RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table B.6.2-7, Table 6.2-8). Studies from the submitted dossier including experiments with the active substance (e.g. combination toxicity) are independent of their acceptability listed below and reported in the appropriate chapters of the RAR. However, non-acceptability – in the case of strong deviations from current guidelines – for these studies is noted in the tables. Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table B.6.2-7: Summary of oral acute toxicity studies: beta-cyfluthrin

Endpoint	Vehicle	Species	Sex	Result [mg/kg bw]	Comment	Reference
acute oral	cremophor/water	rat	male	LD_{50} = 16	- document is a summary, no detailed information available (e.g. purity, therefore not acceptable) - observation period only 7 days	[redacted] 1984 (MO-00-013959)[1] TOX2005-1676
acute oral	cremophor/water	rat	male	LD_{50} = 11	- document is a summary, no detailed information available (e.g. purity, therefore not acceptable)	[redacted] 1986 (MO-00-013962)[1] TOX2005-1677
acute oral	cremophor/water	chicken	female	LD_{50} > 5000	- animals not fasted - only one dose	[redacted], 1985 (13689) TOX9402234

[1] Studies are in reference list of applicant.

Table 6.2-8: Summary of oral acute toxicity studies: cyfluthrin

Endpoint	Vehicle	Species	Sex	Result [mg/kg bw]	Comment	Reference
acute oral	cremophor/water	rat	male	LD_{50} = 20	- combination study - LD_{50} (cyfluthrin + propoxur) = 57 mg/kg bw - no necropsy	[redacted], 1984 (12544)[1] TOX9401948
acute oral	cremophor/water	rat	male	LD_{50} = 20	- combination study - LD_{50} (cyfluthrin + dichlorvos) = 70 mg/kg bw - no necropsy	[redacted], 1984 (12567)[1] TOX9401949
acute oral	cremophor/water	rat	male	LD_{50} = 20	- combination study - LD_{50} (cyfluthrin + fenfluthrin) = 67 mg/kg bw - no necropsy	[redacted], 1984 (12572) Z17076
acute oral	cremophor/water	rat	male	LD_{50} = 14.3	- combination study - the part with triflumuron is not acceptable (no purity given, limit test with only one sex) - no necropsy	[redacted], 1982 (10516)[1] TOX9401946

Endpoint	Vehicle	Species	Sex	Result [mg/kg bw]	Comment	Reference
acute oral	cremophor/water	rat	male	LD_{50} = 18	- combination study - LD_{50} (cyfluthrin + methamidphos) = 26 mg/kg bw	[redacted] 1983 (12003)[1] TOX9401947
acute oral	cremophor/water	rat	male	LD_{50} = 15	- combination study - only two doses tested - LD_{50} (cyfluthrin + imidacloprid) = 414 mg/kg bw	[redacted], 1994 (23420 A)[1] TOX2001-1764
acute oral	cremophor/water	rat	male	LD_{50} = 19.6	- study for antidote effect - no necropsy	[redacted], 1983 (11854)[1] TOX9401941
acute oral	PEG 400	rat	male	LD_{50} = 500	- combination study - LD_{50} (cyfluthrin + omethoate) = 218 mg/kg bw	[redacted], 1988 (16968)[1] TOX9401950
acute oral	PEG 400	rat	male female	LD_{50} = 869 LD_{50} = 1271	- animals not fasted	[redacted] 1980 (8800) TOX9401853
acute oral	PEG 400	rat	male female	LD_{50} = 590 LD_{50} = 1189		[redacted] 1980 (8800) TOX9401853
acute oral	acetone/peanut oil	rat	male female	LD_{50} = 155 LD_{50} = 160		[redacted], 1987 (15847) TOX9401862
acute oral	cremophor/water	mouse	female	LD_{50} <100	- preliminary LD_{50} determination - no detailed information given (e.g. doses, group size)	[redacted], 1982 (10931) TOX9401854
acute oral	PEG 400	mouse	male	LD_{50} >5000 (diasteromer I) LD_{50} = 31 (diastereomer II) LD_{50} >5000 (diastereomer III) LD_{50} >5000 (diastereomer IV)	- document is a summary, no detailed information available (e.g. purity, therefore not acceptable)	[redacted], 1980 (99168)[1] TOX9550282

Endpoint	Vehicle	Species	Sex	Result [mg/kg bw]	Comment	Reference
acute oral	PEG 400	mouse	male female	LD_{50} = 291 LD_{50} = 609		[redacted], 1980 (8800) TOX9401853
acute oral	PEG 400	rabbit	male	LD_{50} >1000	- only three animals per dose - no necropsy	[redacted], 1980 (8800) TOX9401853
acute oral	PEG 400	dog	male	LD_{50} >100	- vomiting at 50 mg/kg bw and above - only two animals per dose - no necropsy	[redacted] 1980 (8800) TOX9401853
acute oral	cremophor/water	dog	male female	LD_{50} >100	- vomiting observed - animals not fasted - only two animals per dose (1 per sex) - only two doses - no necropsy	[redacted], 1981 (MO-01-005194) TOX9401856
acute oral	cremophor/water	hen	female	LD_{50} >5000	- animals not fasted - only two doses tested	[redacted], 1985 (R 3621) TOX9401860
acute oral	PEG 400	hen	female	LD_{50} ca. 5000	- animals not fasted	[redacted], 1981 (9753)[1] TOX9401916
acute oral	PEG 400	hen	female	LD_{50} ~ 4500	- animals not fasted - only two doses tested	[redacted], 1985 (3622) TOX9401861

[1] The study is in the reference list of the applicant. A detailed description of the study is given in the appropriate chapters of the RAR. (e.g. detailed information on acute oral combination studies is preseneted in chapter B.6.8.2.3)

Further studies (literature search for cyfluthrin and beta-cyfluthrin)

Data point: KCA 5.2.1

Report: Bhushan, B. et al., 2013, ASB2015-644
Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin, Arh Hig Rada Toksikol (64), 57-67

Deviations: The part concerning LD_{50} determination is from earlier research and refers to a document that is not available. Therefore, detailed information about the animals and the study design are not available.
The animals were not fasted.

GLP: Not applicable.

Acceptability: Supplementary.

Summary:
A study for LD_{50} calculation after oral administration of beta-cyfluthrin (source: Bayer India Ltd., Mumbai, purity: 95 %) in male rats (not fasted) was cited. The LD_{50} value of beta-cyfluthrin in water was reported as 354.8mg/kg bw.

Conclusion:

As no detailed information are available, the publication cannot be evaluated. Under the conditions used in the study and based on the information given in the report, the oral LD_{50} value of cyfluthrin (solvent: water) in male rats is 354.8 mg/kg bw. The study results are considered to represent supplemental information (e.g. no batch number, no individual values reported).
Treatment-related effects in rats observed following single or repeated administration of beta-cyfluthrin are described in section B.6.3 of the RAR (Bhushan, 2010, ASB2015-1098, Bhushan, 2013, ASB2015-644).

B.6.2.2 Dermal

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point: KCA 5.2.2 /01

Report: [redacted] 1987, TOX9550259
FCR 4545 technical - Study of the acute dermal toxicity to rats (formulation in polyethylene glycol E 400).
Report no.: 16179 (November 04, 1987); [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 402 (1981) which complies to Directive 92/69/EEC method B 3.

Deviations: None.
Deviation from current guideline (OECD TG 1987) (only listed if relevant for acceptability decision):
None.

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is acceptable.

(dates of exp. work: August - September 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered dermally for 24 h to Wistar rats (strain Bor:WISW [SPFCpb], source: [redacted]) in the following dosing schedule:
0-100-1000-2500-5000 mg/kg bw, 5 male rats/dosage group,
0-100-1000-2500-5000 mg/kg bw, 5 female rats/dosage group.
The substance was formulated as a paste with PEG 400 and applied within a bandage onto the non-abraded dorsal skin that had been shorn the day before (treated area 5 x 6 cm). The control animals received 0.6 ml/kg bw PEG 400. After removal of the bandages the treated skin was washed with soap and water.
Recording period: 0-14 days.

Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-9: Percutaneous LD_{50} in rats

	Formulation agent	NOAEL (systemic) [mg/kg bw]#	LD_{50} [mg/kg bw]
Male rats	PEG 400	100	>5000
Female rats	PEG 400	100	>5000

= maximum dosage without clinical signs.

Onset of death: On day 4 (one female rat at a dose of 5000 mg/kg bw).
Clinical signs:
Dose of 1000 mg/kg bw: Lethargy, uncoordinated gait, splayed gait, salivation (1 day to a maximum of 8 days),
Dose of 5000 mg/kg bw: difficult breathing, soft faeces (females only, day 3 and 4).
Local skin signs: Incrustations on the treated areas.
Body weight: A slight effect on weight gain during the first few days (all groups including the control group), compensated at day 14.
Gross pathology: Female animal that died on day 4: Lung: mottled, severely distended; gastrointestinal tract: severely distended, almost empty.
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

With regard to the duration of clinical signs the prolonged effects were dose related. The toxic action was reversible during the observation period. Beta-cyfluthrin exhibited minimal toxicity to rats, following acute percutaneous treatment in PEG 400.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable.
This acute dermal toxicity study was performed in accordance with OECD-Guideline no. 402 (adopted in May 12, 1981).
Male and female rats received up to 5000 mg/kg body weight of the test compound (paste with polyethylene glycol E 400). Under the conditions of the study and based on the information given in the report, the dermal LD_{50} value for male and female rats is greater 5000 mg/kg body weight.
Clinical signs were observed from a concentration of 1000 mg/kg body weight (e.g. lethargy, uncoordinated gait, splayed gait) in rats of both sexes. However, animals developed local clinical signs like incrustation on treated area already from a concentration of 100 mg/kg body weight.

Data point: KCA 5.2.2 /02

Report: [redacted] 1987, TOX9550260
FCR 4545 technical - Study of the acute dermal toxicity to rats (formulation with xylene).
Report no.: 16184 (November 05, 1987); [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 402 (1981) which complies to Directive 92/69/EEC method B 3.

Deviations:	The treated skin area was not specified in the report (only in the dossier). Deviation from current guideline (OECD TG 1987) (only listed if relevant for acceptability decision): None.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: August - September 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered dermally for 24 h to Wistar rats (strain Bor:WISW [SPFCpb], source: [redacted]) in the following dosing schedule:
0-100-1000-2500-5000 mg/kg bw, 5 male rats/dosage group,
0-100-1000-2500-5000* mg/kg bw, 5 female rats/dosage group (*4 animals).
The substance was formulated as a paste with xylene and applied within a bandage onto the non-abraded dorsal skin that had been shorn the day before (treated area 5 x 6 cm). The control animals received 0.4 ml xylene. After removal of the bandages the treated skin was washed with soap and water.
Recording period: 0-14 days.
Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Table B.6.2-10: Percutaneous LD_{50} in rats

	Formulation agent	**NOAEL (systemic) [mg/kg bw]#**	**LD_{50} [mg/kg bw]**
Male rats	xylene	100*	>5000
Female rats	xylene	<100	>5000

= maximum dosage without clinical signs which are related to the test substance.
* = 0 mg/kg bw: males having soft feces (day 3 and 4).

Clinical signs: 100 mg/kg bw: Vocalisation and jumping (females only, up to 2 days). Higher doses: Lethargy, uncoordinated gait, splayed gait, vocalisation and jumping, occasionally digging and preening movements, salivation (beginning after ca. 25 min for up to a maximum of 11 days); 5000 mg/kg bw difficult breathing (males only, day 3 for up to day 11).
Local skin signs: Irritations which were comparable in control and substance treated groups.
Body weight: Slight weight reduction in individual animals in all (including control) groups, compensated at day 14.
Gross pathology: Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

The prolonged clinical signs were reversible during the observation period. Beta-cyfluthrin exhibited minimal toxicity to rats, following acute percutaneous treatment in xylene.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section was revised.
This acute dermal toxicity study complied with OECD-Guideline no. 402 (adopted in May 12, 1981).
Male and female rats received up to 5000 mg/kg body weight of the test compound (paste with xylene). Under the conditions of the study and based on the information given in the report, the dermal LD_{50} value for male and female rats is greater 5000 mg/kg body weight.
Soft faeces were observed in the male control group and after treatment with 2500 mg/kg body weight (but in no other dose group). From a concentration of 1000 mg/kg body weight male rats showed other clinical signs (e.g. lethargy, uncoordinated and sprayed gait). In female rats clinical signs like vocalisation and jumping were already observed from a dose of 100 mg/kg body weight.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.2.2 /04
Report:	[redacted] 2005, ASB2014-7721 FCR 4545 - Acute toxicity in the rat after dermal administration. Report No: AT02656 (November 28, 2005), M-263229-01-1; [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 402 (1987).
Deviations:	None.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: October 05, 2005 – October 19, 2005).

Materials and methods:

Beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %) was administered dermally to 5 male and 5 female Wistar rats (strain HsdCpb:Wu, source: [redacted]). Rats received a single dermal dose of 2000 mg/kg bw under semi-occlusive conditions for 24 h.
One day before treatment, the back and flanks of the rats were shorn (approximately 10 % of the body surface area). For each dose and animal the required amount of pure solid test substance was weighed and transferred to a wet gauze-layer (moistened with PEG 400) (6.0 cm × 5.0 cm = 30.0 cm²) of a "Cutiplast® steril" coated with air-tight "Leukoflex®". The gauze strip was placed on the rat's back and secured in place using cohesive tape (8 cm × 23 cm) and additionally covered with a rat jacket, which was connected with a safety pin to the stretch tape to ensure that the animals could not ingest the test substance. After 24 h the dressings were removed and the area was rinsed with tepid water using soap and gently patting the area dry.
Recording period: 0-14 days.
Body weight: Weekly.
Necropsy: All animals (survivors sacrificed by carbon dioxide).
Statistical method: LD_{50} calculation by means of a software program according to Spearman, Kärber (based on method of D. J. Finney, [1971]). Algorithm was based on publication of L. Sachs (1984). If LD_{50} value calculation was not possible by this method, an assessment (considering applied dose and dose-response curve) was made.

Results and discussions:

Table 6.2-11: Percutaneous LD_{50} in rats

	Formulation agent	NOAEL (systemic) [mg/kg bw]#	LD_{50} [mg/kg bw]
Male rats	PEG 400	2000	>2000
Female rats	PEG 400	2000	>2000

= maximum dosage without clinical signs which are related to the test substance.

Mortality/clinical signs/body weight/gross pathology: A dose of 2000 mg/kg bw was tolerated without mortalities, systemic clinical signs (e.g. neurotoxic effects), impact on weight gain and gross pathological findings.
Partial reddening and partial encrustation of the treatment area were the only clinical signs noted.

Conclusion:

Under the conditions of OECD-Guideline no. 402 (adopted in February 24, 1987) acute dermal toxicity was investigated. The study is considered acceptable.
Male and female rats received 2000 mg/kg body weight of the test compound (wet gauze layer moistened with PEG 400). Under the conditions of the study and based on the information given in the report, the percutaneous LD_{50} value of the test compound is greater 2000 mg/kg body weight.
Some treated animals developed local clinical signs like partly encrustation or partly reddening (only females) at 2000 mg/kg bw.

Further studies available to the RMS:

The studies listed below were not submitted by the applicant for renewal of approval. However, the studies are available to the RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table 6.2-12). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table 6.2-12: Summary of dermal acute toxicity studies: cyfluthrin

Endpoint	Vehicle	Species	Sex	Result [mg/kg bw]	Comment	Reference
acute dermal	cremophor/water	rat (24 h contact)	male female	LD_{50} > 5000	- only two doses tested (limit test not sufficient) - unclear which sex was used at lower concentration - no necropsy	[redacted] 1982 (10931) cited in [redacted], 1985 TOX9401854
acute dermal	PEG 400	rat (24 h)	male female	LD_{50} > 5000	- no neropsy - no detailed information given	[redacted] 1982 (10931) TOX9401854

acute dermal	physiological NaCl solution	rat (24 h contact)	male female	LD_{50} > 5000	- only two doses tested (limit test not sufficient) - unclear which sex was used at lower concentration - no necropsy	[redacted] 1982 (10931) cited in [redacted] e, 1985 TOX9401854

For beta-cyfluthrin no further studies were available.

B.6.2.3 Inhalation

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point: KCA 5.2.3 /01

Report: [redacted] 1985, TOX9550261
FCR 4545 (techn.) - Study for acute inhalation toxicity.
Report no.: 13751 (August 20, 1985); [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 403 (1981) which complies to Directive 92/69/EEC method B 2.

Deviations: None.
Deviation from current guideline (OECD TG 2009) (only listed if relevant for acceptability decision):
None.

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is acceptable.

(Dates of exp. work: May - July 1985).

Materials and methods:

Groups of 5 male and 5 female Wistar rats (strain BOR:WISW [SPFCpb], source: [redacted]) received beta-cyfluthrin (batch no: 16002/84, purity: 98.5 %) via inhalation (head-nose only). The substance was administered as an aerosol (dynamic spraying) or as dust during 4 h each. The formulating agent was ethanol/polyethylene glycol E 400 (1:1). The dosing schedule was as follows:
Aerosol: Nominal concentrations 0 (vehicle) and 10-50-100-300-500-700-1000 mg/m³ air corresponding to analytical concentrations of 1.4-7.3-11.1-44.6-80.2-100.9-193.2 mg/m³ air.
Dust: Nominal concentrations 0 (air) and 246-499-967 mg/m³ air corresponding to analytical concentrations of 198.8-484.0-840.8 mg/m³ air.
Recording period: 0-14 days.
Body weight: Day 0, 7, 14.
Necropsy: All animals (survivors sacrificed by Evipan).
Statistical method: LC_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Analysis of test atmosphere: Aerosol: Inhalable particle content ca. 100 % (particles ≤5 µm). Dust: Inhalable particle content ca. 50 % (particles ≤5 µm):

Table B.6.2-13: Inhalative LC_{50} in rats

	Formulation agent	NOAEC [mg/m³]#	LC_{50} [mg/m³]
Male rats (4 h, aerosol)	ethanol/ PEG 400	<1.4	approx. 90
Female rats (4 h, aerosol)	ethanol/ PEG 400	<1.4	approx. 100
Male rats (4 h, dust)		<198.8	approx. 967
Female rats (4 h, dust)		<198.8	approx. 695

= maximum dosage (analytically determined) without clinical signs.

Onset of death: Within 4 to 5 h up to 24 h.
Clinical signs - aerosol: 1.4 and 7.3 mg/m³ air (analytical): reduced motility, bristling, ungroomed coat, dyspnoea; from group 11.1 mg/m³ air (analytical) onward: same symptoms and/or staggering gait, impaired co-ordination of movement, choreoathetotic movements, prostration on stomach, digging and grooming movements, tremor, hyperaemia of the visible nasal mucosa. The signs lasted in most cases for 4-7 h.
Clinical signs - dust: 198.8 mg/m³ air (analytical): reduced motility, bristling and ungroomed coat, dyspnoea, non-specific disturbed behaviour and reddened noses; from group 484 g/m³ air (analytical) onward: same symptoms and/or staggering gait, impaired co-ordination of movement, digging and grooming movements and swollen eyelids, dyspnoea, choreoathetotic movements, tremor and clear hyperaemia of the visible nasal mucosa with severe peri-orbital swelling. The signs lasted from 4 h up to a maximum of 2 days.
Body weight: No effect.
Gross pathology (rats dying intercurrently):
Nose: discoloured reddish; lung: distinctly distended and after dust application reddish and with hepatoid appearance, oedamatous and litter in the oesophagus; kidney: pale; liver: with lobular pattern; spleen: pale; glandular stomach: reddened and after dust application haemorrhagia and ulceroid foci; gastrointestinal tract: yellowish mucous content.
Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

Beta-cyfluthrin had a very high acute toxicity when inhaled as an aerosol (higher inhalable) and a high acute toxicity when inhaled as dust (lower inhalable).

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the results and discussions section was revised.
This acute inhalation study conformed generally to OECD-Guideline no. 403 (adopted in May 12, 1981).
Male and female rats were head-nose exposed to either aerosol (1:1 mixture of ethanol and polyethylene glycol E 400) or dust of the test compound up to an analytical concentration of 193.2 mg/m³ air or 840.8 mg/m³ air, respectively. Under the conditions of the study and based on the information given in the report, the LC_{50} value for the aerosol after 4 h exposure is approx. 90 mg/m³ air for male rats

and approx. 100 mg/m³ air for female rats. The LC_{50} values for the application of dust after 4 h exposure amounts to approx. 967 mg/m³ air or approx. 695 mg/m³ air for male and female rats, respectively.
Clinical signs were observed already from the lowest dose (analytical concentration of 1.4 mg/m³ air for aerosol and 198.8 mg/m³ air for dust). Animals receiving this dose showed for example reduced motility, bristling and dyspnoea.

Data point:	KCA 5.2.3 /02
Report:	[redacted] 1988, TOX9550264 FCR 4545 (c.n.: Cyfluthrin K+L proposed) - Studies for acute inhalation toxicity to the rat to OECD-Guideline no. 403. Report no.: 16911 (July 18, 1988); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 403 (1981) which complies to Directive 92/69/EEC method B 2.
Deviations:	None. Deviation from current guideline (OECD TG 2009) (only listed if relevant for acceptability decision): None.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: February - March 1988).

Materials and methods:

Groups of 5 male and 5 female Wistar rats (strain BOR:WISW [SPFCpb], source: [redacted]) received beta-cyfluthrin (batch no: 16001/87, purity: 97.9 %) via inhalation (head-nose only). The substance was administered as an aerosol (dynamic spraying) or as dust during 4 h each. The formulating agent was ethanol/polyethylene glycol E 400 (1:1). The dosing schedule was as follows:
Aerosol: Nominal concentrations 0 (vehicle) and 500-700-850-1000 mg/m³ air corresponding to analytical concentrations of 53.4-80.5-82.2-96.7 mg/m³ air.
Dust: Analytical concentrations 0 (air) and 212-417-497-640-867 mg/m³ air.
Recording period: 0-14 days incl. recording of reflexes (cornea, pirmal, myotactic, light, startle and external stimuli [noises]).
Body weight: Day 0, 3, 7, 14.
Necropsy: All animals (survivors sacrificed by Evipan).
Statistical methods: LC_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983). For the other parameters different methods (Chi-square-test, Fisher-test, Variance analysis (ANOVA), Box's test, Tukey-Kramer-test, modified by Games and Howell).

Results and discussions:

Analysis of test atmosphere: Aerosol: Inhalable particle content ca. 100 % (particles ≤5 µm). Dust: Inhalable particle content 55-64 % (particles ≤5 µm).

Table B.6.2-14: Inhalative LC_{50} in rats

	Formulation agent	NOAEC [mg/m³]#	LC_{50} [mg/m³]*
Male rats (4 h, aerosol)	ethanol/ PEG 400	<53.4	approx. 82
Female rats (4 h, aerosol)	ethanol/ PEG 400	<53.4	81 (62-89)
Male and female rats (4 h, dust)		<212	532 (472-600)

= maximum dosage (analytically determined) without clinical signs.
* = () confidence interval (95 %).

Onset of death: Aerosol: <4 h. Dust: Day 0 to day 4.
Clinical signs: Aerosol: From 53.4 mg/m³ air (analytical) reduced motility, tonic cramps, bradypnoea, reduced reflexes, chromodacryorrhoea, bloody nose, piloerection, unkempt fur; from 80.5 mg/m³ and 82.3 mg/m³ air (analytical) additionally rolling over, at 96.7 mg/m³ air (analytical) 100 % mortality (length of signs: <4 h up to a maximum of 2 days).
Dust: From 212 mg/m³ air (analytical) reduced motility, staggering gait, red nose, unkempt fur, bradypnoea; from 417 mg/m³ air (analytical) in addition reduced reflexes as well as for females in addition rolling over and tonic extension spasms; 497 mg/m³ air (analytical) in addition respiratory distress; at 640 mg/m³ in addition prostration on stomach; at 867 mg/m³ air (analytical) 100 % mortality (length of signs: <4 h to 3 days).
Body weight: Marginal, but toxicologically insignificant effect observed in group receiving 212 mg/m³ air (analytical). Clinically relevant effect on weight gains in rats receiving 640 mg/m³ air (analytical).
Gross pathology: Rats dying intercurrently: Lung: distended, of hepatoid appearance, oedamatous; kidney: pale (after aerosol application); liver: pale, with lobular pattern; gastrointestinal tract: reddened and after dust application with yellowish to bloody mucous content. Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.

Conclusion:

Beta-cyfluthrin had a very high acute toxicity when inhaled as an aerosol (higher inhalable) and a high acute toxicity when inhaled as dust (lower inhalable).

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the results and discussion section were revised.
This acute inhalation study is in accordance with OECD-Guideline no. 403 (adopted in May 12, 1981). Male and female rats were head-nose exposed to either aerosol (1:1 mixture of ethanol and polyethylene glycol E 400) or dust of the test compound up to an analytical concentration of 96.7 mg/m³ air or 867.0 mg/m³ air, respectively. Under the conditions of the study and based on the information given in the report, the LC_{50} value for the aerosol after 4 h exposure is approx. 82 mg/m³ air for male rats and 81 mg/m³ air for female rats. The LC_{50} values for the application of dust after exposure for 4 h is 532 mg/m³ air for male and female rats collated.
Clinical signs were observed already from the lowest dose onward (analytical concentration of 53.4 mg/m³ air for aerosol and 212 mg/m³ air for dust). Male and female rats exposed to the test compound of this dose showed for example bristling coat (in case of aerosol) or ungroomed coat (in case of dust), respectively.

Further studies available to RMS

The studies listed below were mostly not submitted by the applicant for renewal of approval (exceptions are marked in the table). However, the studies are available to RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table 6.2-15). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table 6.2-15: Summary of inhalative acute toxicity studies: cyfluthrin

Endpoint	Vehicle	Species	Sex	Result [mg/m³]	Comment	Reference
acute inhalative	ethanol:PEG 400 (1:1), aerosol	rat (1-hour exposure, nose only)	male female	LC_{50} > 1089	- inhalable particle content not given - no vehicle control	[redacted], 1980 (8800) TOX9401853
acute inhalative	ethanol:PEG 400 (1:1), aerosol	rat (4-hour exposure, nose only)	male female	LC_{50} = 469-592	- inhalable particle content not given - no vehicle control	[redacted], 1980 (8800) TOX9401853
acute inhalative	ethanol:PEG 400 (1:1), aerosol	rat (4-hour exposure, probably head/nose only)	male female	LC_{50} = 1010 LC_{50} = 1020	- inhalable particle content not given	[redacted], 1984 (269) TOX9401866
acute inhalative	ethanol:PEG 400 (1:1), aerosol	rat (4-hour exposure, head/nose only)	male female	LC_{50} = 405	- no vehicle control	[redacted], 1987 (15612) TOX9401867
acute inhalative	water DMSO:PEG 400 aerosol	rat (4-hour exposure, head/nose only)	1) male female 2) male female	LC_{50} > 735 LC_{50} = 200-735 LC_{50} =575 LC_{50} =490	- inhalable particle content not given - no vehicle control	[redacted], 1982 (10965) TOX9401864
acute inhalative	ethanol:PEG 400 (1:1), aerosol	rat (5 x 6 hour exposure, nose only)	male female	LC_{50} = 47-196	- inhalable particle content not given - no vehicle control - no different time points	[redacted], 1980 (8800) TOX9401853
acute inhalative	ethanol:PEG 400 (1:1), aerosol	mouse (4-hour exposure, head/nose only)	male female	LC_{50} ~ 141		[redacted], 1989 (17765) TOX9401871

acute inhalative	ethanol:PEG 400 or water/cremophor, aerosol	hen (4-hour exposure, whole body)	female	LC_{50} >596	- inhalable particle content not given - different solvents - no vehicle control	[redacted], 1983 (11558)[1] TOX9401865

[1] The study is in the reference list of the applicant. A detailed description of the study is given in the appropriate chapters of the RAR.

For beta-cyfluthrin no further studies were available.
Studies considering other routes for LD_{50} determination are presented in section B.6.8 of the RAR.

B.6.2.4 Skin irritation

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point: KCA 5.2.4 /01

Report: [redacted] 1985, TOX9550265
FCR 4545 (techn.) - Study for irritant/corrosive effect on skin and eye (rabbit).
Report no.: 13707 (August 09, 1985); [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 404 (1981) which complies to Directive 92/69/EEC method B 4.

Deviations: Test substance was not moistened before treatment of animals (recommended in OECD 1981).
Deviation from current guideline (OECD TG 2002) (only listed if relevant for acceptability decision):
None.

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered not acceptable.

(dates of exp. work: April - May 1985).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 98.5 %) was administered dermally for 4 h to one female and two male rabbits (strain HC:NZW, source: [redacted]). The dose was 500 mg/animal. The substance was applied within a Hansamed ″Hypoallergen″ dressing (Beiersdorf no. 2342) onto a flank skin area from 6 x 6 cm (shorn the day before). A further dressing was moistened with water and placed on the opposite flank area. After removal the treated skin was washed with water. Recording of skin reactions (erythema, eschar formation and edema): 1, 24, 48, 72 h, 7 days p.a.; scoring according to Draize.

Results and discussions:

Table B.6.2-16: Test for skin irritant effects in rabbits (notified by [redacted])

Animal no. (sex)	grade after								Mean value after	
	24 h		48 h		72 h		7 days		24 h, 48 h, 72 h	
	E#	O*	E	O	E	O	E	O	E	O
M7 (female)	1	0	1	0	0	0	0	0	0.7	0
L14 (male)	1	0	1	0	0	0	0	0	0.7	0
L15 (Male)	1	0	1	0	0	0	0	0	0.7	0

E = erythema and eschar formation.
* O = oedema formation.

The mean values for erythema/eschar or oedema formation were 0.7 or 0, respectively. The irritant effects observed were reversible within the observation period.

Conclusion:

The substance was not irritating to the skin.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be acceptable. However, due to deviations from the current test guideline listed below the study is now considered to be no longer acceptable. It should be noted that the results and discussions section was revised.
This study was based on OECD-Guideline no. 404 for acute dermal irritation/corrosion (adopted in May 12, 1981).
The study was performed with rabbits.
Guideline no. 404 advises to moisture the test substance with water or another vehicle in order to ensure good contact with the skin. However, wetting of the solid test compound is not mentioned in the report. Therefore, the study is considered not acceptable.
Under the conditions of the study and based on the information given in the report, the mean values in all animals from grading's at 24, 48 and 72 h after treatment amount to 0.7 for erythema and eschar formation and 0 for oedema formation. All effects observed were reversible.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.2.4 /04
Report:	Vohr, H.-W. 2005, ASB2014-7722 FCR 4545 Project: Beta-cyfluthrin technical - Evaluation of corrosive properties by using an artificial 3D-Skin model. Report no.: AT02855 (November 30, 2005), M-268673-01-1; Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany
Guideline(s):	The test was run according to OECD-Guideline no. 431 draft (2004).

Deviations:	No positive control data were reported. No validation of the employed test system is documented. No reference to historical data was mentioned. No single data are presented. Incubation time with MTT not according to the guideline. (all listed deviations concern recommendations in OECD 2004) Deviation from current guideline (OECD TG 2014) (only listed if relevant for acceptability decision): None.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is not acceptable.

(date of exp. work: October 25, 2005).

Materials and methods:

The corrosive potential of beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %) was tested using reconstructed human epidermis EST-1000 (RHS, source: CellSystems, St. Katharinen, Germany). A 100 % concentration was tested on the skin/epidermal equivalents in triplets.
The test item was applied at a 100 % concentration i.e. 25 mg per insert, (plus 50 µL 0.9 % NaCl to moisten and ensure good contact with the skin) for 3 min (RT) and 60 min in the incubator (3 inserts per period of incubation time), respectively. 0.9 % NaCl (50 µL) treated epidermal models were used as negative controls (determination in triplicates).
After the incubation period the inserts were washed carefully in PBS (3 times each insert) and the MTT viability test was performed.

Results and discussions:

Table B.6.2-17: MTT test of cell viability for investigation of corrosive potency

Test substance	Cell viability after	
	3 min [%]	**60 min [%]**
Beta-cyfluthrin	98.56	87.00
Negative control	100	100

The MTT method has determined following values of viability after 3 or 60 min of incubation: 98.56 % and 87.00 %, respectively. Thus, the results show that no corrosive property of the test item was determined by the assay used. Substances are classified as “corrosive” in this test system, if the cell viability after 3 min of incubation to the test item is decreased by more than 50 %, or if the cell viability after 60 min of exposure to the test item is less than 15 %.
As can be seen from the information in the table below the test item beta-cyfluthrin was not detected as positive (exceeding the LD_{50} value and the 15 % viability value, respectively) by the EST-1000 model after an incubation period of 3 min or 60 min.

Conclusion:

The study on skin corrosion (3D-Skin model) of beta-cyfluthrin complied with OECD-Guideline no. 431 (adopted in April 13, 2004) even though it is unclear why a draft was used.
The study is considered non-acceptable.
The main reason for this decision is the absence of a ‘concurrent’ positive control. Such a control is not mentioned in the report even though this should be the basic requirement for ensuring reliability of the study.
In addition to this, there are further deviations from the test guideline (e.g. incubation with MTT for 2

instead of 3 h) and the study report is incomplete (neither historical data nor individual data are presented).

Data point:	KCA 5.2.4 /05
Report:	[redacted]. 2005, ASB2014-7723 FCR 4545 - Acute Skin Irritation/Corrosion on Rabbits. Report No: AT02655 (November 25, 2005), M-263238-01-1; [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 404 (2002).
Deviations:	Individual body weights of the animals at the conclusion of the test were not reported (demanded in OECD TG 2002).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: November 2, 2005 – November 5, 2005).

Materials and methods:

The skin-irritating potential of beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %) was tested in 3 female albino rabbits (strain Esd:NZW; source: [redacted].
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. The body weight of each animal was recorded at the beginning of the study.
0.5 g of the pulverised test substance moistened with water was applied to the skin of the animal under a gauze patch. The treated skin area was approx. 2.5 x 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. Thus, access by the animal to the patch and resultant ingestion of the test substance was prevented.
After the exposure period the dressing and patch were removed. The exposed skin area was carefully washed with water. The surrounding untreated skin served as control.
In the first step, only one animal was exposed to the test substance; three test patches were applied successively to this animal. The first patch was removed after three minutes. As no serious skin reactions were observed, the second patch was removed after 1 h. At this stage the exposure was extended to 4 h. After 4 h, the patch was removed and the application site was carefully washed with water. Responses were graded one hour later. The test was completed using two additional animals, exposed for 4 h.
Dermal irritation was scored approximately at 1, 24, 48 and 72 h after patch removal. Since no irritation indices were observed after 72 h, the study was finished at this point.
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.

Results and discussions:

Table B.6.2-18: Test for skin irritant effects in rabbits (notified by Schüngel, 2005, ASB2014-7723)

Animal no.	grade after								Mean value after	
	24 h		48 h		72 h		7 days		24 h, 48 h, 72 h	
	E#	O*	E	O	E	O	E	O	E	O
1	0	0	0	0	0	0	n.d.	n.d.	0	0
2	0	0	0	0	0	0	n.d.	n.d.	0	0
3	0	0	0	0	0	0	n.d.	n.d.	0	0

E = erythema and eschar formation, n.d. = not determined.
* O = oedema formation, n.d. = not determined.

Neither animal showed erythema or oedema at the application or control site. There were no systemic intolerance reactions.

Conclusion:

This acute dermal irritation/corrosion test with rabbits was in accordance with OECD-Guideline no. 404 (adopted April 24, 2002). The study is considered acceptable.
It should be mentioned that the individual body weights of the animals at the conclusion of the test – as required in OECD-Guideline no. 404 – were not presented in the study report.
Under the conditions of the study and based on the information given in the report, there is no evidence for irritation as all mean scores for erythema (redness) and eschar formation as well as for oedema formation were 0.

Further studies available to RMS

The studies listed below were not submitted by the applicant for renewal of approval. However, the studies are available to RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table 6.2-19). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table 6.2-19: Summary of skin irritation studies: cyfluthrin

Endpoint	Vehicle	Test system	Result	Comment	Reference
skin irritation	none	rabbit (female)	negative	- FCR 1272 used in melted state - observation period only 3 days (TG 404, 1981) - only 100 µl instead of 500 µl tested (TG 404, 1981) - 24 h instead of 4 h exposure (TG 404, 1981) - skin observed after 24 h and 72 h (not 48 h) (TG 404, 1981)	[redacted] 1982 (MO-01-004694) TOX9401872

skin irritation	unclear	rabbit (sex unclear)	negative	- 24 h instead of 4 h exposure (TG 404, 1981) - material section refers to document which is not available - some details remain unclear (e.g. whether substance is moistened)	[redacted], 1980 (8800) TOX9401853

For FCR 4545 no further studies were available.

B.6.2.5 Eye irritation

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.5 /01
Report:	[redacted] 1985, TOX9550265 FCR 4545 (techn.) - Study for irritant/corrosive effect on skin and eye (rabbit). Report no.: 13707 (August 09, 1985); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 405 (1981) which complies to Directive 92/69/EEC method B 5.
Deviations:	None. Deviation from current guideline (OECD TG 2012) (only listed if relevant for acceptability decision): None.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(dates of exp. work: April - May 1985).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 98.5 %) was administered in a dose of ca. 65 mg/animal (100 µl) into the conjunctival sac of one eyelid of three male rabbits (strain HC:NZW, source: [redacted]). The other eye was not treated and therefore used as control. After 24 h the treated eye was rinsed with physiological NaCl solution.
Recording of eye reactions: 1, 24, 48, 72 h, 7 days p.a.; scoring according to Draize and in addition any other findings not covered by the Draize scale.

Results and discussions:

Table B.6.2-20: Test for irritant/corrosive impact of the test compound on the rabbit's eye (Pauluhn)

Animal no.	grade after#																Mean value after			
	24 h				48 h				72 h				7 days				24 h, 48 h, 72 h			
	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE	CO	IR	CR	COE
J1	0	0	2	2	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1.3	1
M27	0	0	2	1	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1.3	0.7
M24	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0.7	0.3

CO = corneal opacity, IR = iritis, CR = conjunctival redness, COE = conjunctival oedema.

There were slightly irritating effects especially after 1 h and 24 h to the conjunctivae (redness, swelling, tear flow). Considering the time points 24, 48 and 72 h, the mean values for corneal opacity and iritis were 0 and for all conjunctival parameters not above 1.3.

Conclusion:

Beta-cyfluthrin showed a slightly irritating effect on the eye. But according to the EC criteria, the substance is not to be classified as irritating to eyes.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the results and discussions section was revised.
This study was based on OECD-Guideline no. 405 for acute eye irritation/corrosion (adopted in May 12, 1981).
The study was performed with rabbits.
It remains unclear whether the solid test substance – as advised in the test guideline – was ground to fine dust before treating the animals.
Under the conditions of the study and based on the information given in the report, the mean scores in all animals following grading at 24, 48 and 72 h after animal treatment at 24, 48 and 72 h after treatment are 0 for cornea opacity and iritis, ≤1.3 for conjunctival redness and ≤1.0 for conjunctival swelling. Furthermore, all effects observed were reversible.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.2.5 /02

Report: [redacted] 2005, ASB2014-7724
FCR 4545 – Acute Eye Irritation on Rabbits.
Report No: AT02657 (November 25, 2005), M-263232-01-1; [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 405 (2002).

Deviations: The method used for examination of eye irritation is not specified in the report (demanded in OECD TG 2002).
Deviation from current guideline (OECD TG 2012) (only listed if relevant for acceptability decision):
None.

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is acceptable.

(dates of exp. work: November 4, 2005 – November 7, 2005).

Materials and methods:

Three female albino rabbits (strain HsdIf:NZW, source: [redacted]) were used in this study. 0.1 g of the test substance (batch no.: FFEBCTQ043, purity: 99.2 %) were pulverised and instilled into the conjunctival sac of one eye of the first animal. The eye was not rinsed for at least 24 h following instillation. The other eye, which remained untreated, served as control. Since 1 h after treatment a severe irritation was not observed, two further rabbits were treated in the same fashion.
Eye irritations were scored and recorded approx. at 1, 24, 48 and 72 h post application. Since no irritation indices were observed at 72 h, the study was finished at this point.
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.

Results and discussions:

Table B.6.2-21: Test for irritant impact of the test compound on the rabbit's eye (Schüngel)

Animal no.	grade after#												Reversible after				Mean value after			
	24 h				48 h				72 h				days				24 h, 48 h, 72 h			
	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC	CO	IR	CR	CC
1	0	0	2	1	0	0	1	0	0	0	0	0	n.a.	n.a	3	2	0	0	1	0.3
2	0	0	1	0	0	0	0	0	0	0	0	0	n.a	n.a	2	1*	0	0	0.3	0
3	0	0	2	1	0	0	0	0	0	0	0	0	n.a	n.a	2	2	0	0	0.7	0.3

CO = corneal opacity, IR = iritis, CR = conjunctival redness, CC = chemosis conjuctivae, n.a. = not applicable.
* = in respect of the result 1 h post application.

The control eyes did not show any abnormal findings. There were no systemic intolerance reactions.
All animals showed conjunctival erythema and 2/3 animals chemosis (Grade 1-2) after 24 h post application. In one animal grade-1 conjunctival erythema persisted until 48 h post application. None of the animals showed signs of eye irritation at 72 h post application. Iris and cornea were not affected by treatment at any time point. Thus, beta-cyfluthrin is not irritating to eyes.

Conclusion:

Eye irritation in rabbits was investigated in compliance with OECD-Guideline no. 405 (adopted in April 24, 2002). The study is considered acceptable.
However, the method used for examination of eye irritation - which is required according to the test guideline - is not specified in the report.
Under the conditions of the study and based on the information given in the report, there was no evidence for irritation as the mean scores of corneal opacity and iritis were 0 and the maximal mean scores of conjunctival redness and oedema were 1.0 and 0.3, respectively (Regulation (EC) No. 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures).

Further studies available to RMS

The studies listed below were not submitted by the applicant for renewal of approval. However, the studies are available to RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table 6.2-22). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table 6.2-22: Summary of eye irritation studies: cyfluthrin

Endpoint	Vehicle	Test system	Result	Comment	Reference
eye irritation	none	rabbit (female)	positive[1]	- FCR 1272 used in melted state - only 7 days observed (up to 21 according TG 405, 1981) - irrigation after 30 s or no irrigation (24 h exposure according TG 405, 1981)	[redacted] 1982 (MO-01-004694) TOX9401872
eye irritation	unclear	rabbit (sex unclear)	positive[2]	- 5 min or 24 h exposure - material section refers to document which is not available - some details remain unclear (e.g. whether gradings are comparable with today's perspective)	[redacted] 1980 (8800) TOX9401853

[1] From the data given it remains unclear whether from the today's perspective the outcome would be positive, too.
[2] If gradings are comparable with today, the substance would be considered as not irritating to eyes (based on mean scores after 24, 48, 72 h).

For FCR 4545 no further studies were available.

B.6.2.6 Skin sensitisation

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.6 /01
Report:	[redacted] 1986, TOX9550266 FCR 4545 techn. - Study for skin sensitising effect on guinea pigs. Report no.: 14426 (March 4, 1986) and 14426 A (Vohr, H. W., February 17, 1994); [redacted]
Guideline(s):	Method recommended by Magnusson and Kligman and in ″Identification of contact allergens in Allergic contact dermatitis in the guinea pig″, ed. C. C. Thomas 102-103 (1970). There were no major deviations from Directive 92/69/EEC Method B 6.
Deviations:	Absence of a reasonable positive control for ensuring reliability of the study (recommended in OECD TG 1981). Dose choice after dose-range-finding study inconclusive (deviation from recommendation in OECD TG 1981). Deviation from recommendation in current guideline (OECD TG 1992) (only listed if relevant for acceptability decision): The test intended as reliability experiment in the addendum is older than 6 months compared to the in life period of the main experiment.
GLP:	The tests followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is not acceptable.

(dates of exp. work: July - August 1985).

Materials and methods:

Male guinea pigs (strain Bor: DHPW [SPF], source: [redacted]) were treated after the following dosing schedule (doses found out in a preliminary range-finding test):
Group 1 (20 animals):
Day 1: Intracutaneous injections (one injection with Freund's complete adjuvant, two injections with 1 % beta-cyfluthrin [batch no: 16002/84, purity: 98.5 %], formulated in Cremophor EL/water with/without Freund's complete adjuvant),
Day 7: Topical application onto the injection sites for 48 h (hypoallergenic dressing (2 x 4 cm) soaked with 25 % beta-cyfluthrin in Cremophor EL/water), 24 h before application the injection sites were shorn and irritation was induced with 10 % sodium lauryl sulphate (formulated in paraffin oil),
Day 21 (challenge-reaction): One dressing with 25 % beta-cyfluthrin in Cremophor EL/water on the left flank and another dressing without the active substance on the right flank for comparison.
Group 2 (control group, 10 animals):
Day 1 and day 7: The same treatment like group 1, but without any active substance,
Day 21: Same treatment like group 1.
Skin inspection: 24 and 48 h after removal of the dressings for challenge-reaction.

Results and discussions:

The incidence (group 1: 2/20 animals (10 %); group 2: 1/10 animals (10 %)) and the intensity of signs (slight redness) were similarly distributed in both groups. The reliability of the test was confirmed by formaldehyde as positive control substance.

Conclusion:

Beta-cyfluthrin did not show a skin-sensitising effect.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be acceptable. However, due to deviations from the current test guideline listed below the study is now considered to be no longer acceptable. It should be noted that the materials and methods section was revised.
According to the report, this test for skin sensitisation in guinea pigs was based on a method recommended by Magnusson and Kligman. Nevertheless, the test procedure is generally in accordance with the OECD-Guideline no. 406 (adopted in May 12, 1981) which already existed when the study was performed.
The main reason for the decision against acceptability is the absence of a reasonable positive control for ensuring reliability of the study. In contrast to the test guideline requirement the study report did not contain a reliability experiment. An addendum contains information on an experiment using formaldehyde as test substance. It remains unclear whether the application of this control (in comparison to other substances with mild-to-moderate skin sensitisation properties, e.g. hexyl cinnamanic aldehyde or mercaptobenzothiazole) was appropriate. Furthermore, experimental details such as the number of animals used in the reliability check are not mentioned. As the animals in the treated group (75 % after first and 80 % after second challenge) as well the animals in the control group (40 % after first challenge and 30 % after second challenge) reacted with skin changes, the study is not evaluable.
Additionally, it seems this latter experiment is older than 1 year compared to the in life period of the main experiment. The current test guideline no. 406 (adopted in July 17, 1992) requires a positive control experiment all 6 months.
Finally, it remains unclear, why the range-finding study was not extended to higher concentrations (above 1 % for intra-dermal induction or above 25 % for topical induction and challenge) in order to investigate possible skin irritating effects at higher concentrations.

Data point:	KCA 5.2.6 /02
Report:	[redacted]. 1994, TOX9550241 FCR 4545 - Study for the skin sensitisation effect in guinea pigs (maximisation test of Magnusson and Kligman). Report no.: 23539 (December 09, 1994); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 406 (1992) which complies to Directive 92/69/EEC method B 6.
Deviations:	The reliability experiment is older than 6 months compared to the in life period of the main experiment (deviation from recommendation in OECD TG 1992). Reason for dose selection after dose-range-finding study unclear (deviation from recommendation in OECD TG 1992).
GLP:	The tests followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is not acceptable.

(dates of exp. work: July - August 1994).

Materials and methods:

Male guinea pigs (strain Hsd/Win:DH, previously termed Bor: DHPW, [SPF]), source: [redacted]) were treated after the following dosing schedule (doses found out in a preliminary range-finding test):
Group 1 (20 animals):

Day 1: Intracutaneous injections (one injection with Freund's complete adjuvant, two injections with 5 % beta-cyfluthrin (batch no: 380466003, purity: 98.6 %), formulated in Cremophor EL/water, 20 mg/animal with/without complete Freund's adjuvant),
Day 7: Topical application onto the injection sites for 48 h (hypoallergenic dressing (2 x 4 cm) soaked with 50 % beta-cyfluthrin in Cremophor EL/water, 250 mg/animal), on the day prior to treatment the injection sites were shorn and irritation was induced with 10 % sodium lauryl sulphate (formulated in vaseline),
Day 21 (challenge-reaction): One dressing with 25 % (125 mg/animal) and one dressing with 50 % (250 mg/animal) beta-cyfluthrin in Cremophor EL/water on the left flank of each animal and another dressing without the active substance on the right flank for comparison.
Group 2 (control group, 10 animals):
Day 1 and day 7: The same treatment like group 1, but without any active substance,
Day 21: Same treatment like group 1.
Skin inspection: 24 and 48 h after removal of the dressings for challenge-reaction.

Results and discussions:

The values after the scoring system were for all animals of both groups 0 (no findings). In an older study animals treated with 2-mercaptobenzothiazole showed skin reactions upon challenge.

Conclusion:

Beta-cyfluthrin did not show a skin-sensitising effect.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be acceptable. However, due to deviations from the current test guideline listed below the study is now considered to be no longer acceptable. It should be noted that the materials and methods section and the results and discussion section were revised.
This Maximisation Test of Magnusson and Kligman for skin sensitisation in guinea pigs was run on the basis of OECD-Guideline no. 406 (adopted in July 17, 1992).
The study cannot be accepted as the methodological reliability tests were performed in July 1993 or Mai 1992 – one to two years before performing the main experiment. This time interval is not in agreement with the current test guideline which requires a positive control experiment all 6 months.
Before performing the main experiment, dose-range-finding studies were performed in order to find the dose for sensitisation induction and challenge. OECD-Guideline no. 406 advises the highest dose to cause mild irritation for the induction exposure. For challenge exposure the highest non-irritating dose should be applied. Since no skin reactions were observed in the whole pilot study, the highest tested concentrations were chosen (5 % for intradermal induction, 50 % for topical induction, 50 % or 25 % for challenge). Therefore, it remains unclear – even though sodium lauryl sulphate was applied in the main study to provoke a local irritation – why the dose-range-finding study was not extended to higher concentrations to investigate possible skin irritating effects induced by higher concentrations.
Under the conditions used in the study and based on the information given in the report, the test compound did not induce dermal redness following challenge exposure (all scores for the control and the test substance group were 0).

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.2.6 /03
Report:	[redacted]. 2005, ASB2014-7725 FCR 4545 – (Project: Beta-cyfluthrin technical) - Study for the Skin Sensitisation Effect in Guinea Pigs (Buehler Patch Test). Report No: AT02683 (November 30, 2005), M-263247-01-1; [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 406 (1992).
Deviations:	Reason for dose selection after dose-range-finding study unclear (deviation from recommendation in OECD TG 1992). Occlusive conditions were neither claimed nor documented for the main study (deviation from recommendation in OECD TG 1992). Buehler test was conducted with three applications – compared with 9 applications which is usually recommended (EFSA Handbook for the experts' meetings, Section 2: Mammalian toxicology, 2010). Stability and homogeneity not tested at relevant concentrations.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is considered supplementary.

(dates of exp. work: October - November 2005).

Materials and methods:

The skin-sensitising potential of beta-cyfluthrin (batch no.: FFEBCTQ043, purity: 99.2 %) was tested in a Buehler test on female guinea pigs (strain Crl:HA, source: [redacted]). Alpha-hexyl cinnamic aldehyde was used for reliability check (30 % for induction, 20 % for challenge, vehicle cremophor EL / sterile physiological saline solution 2 % (v/v)).
In a pilot study on 2 animals, a concentration of 66 % test substance in cremophor EL / sterile physiological saline solution 2 % (v/v) was established as concentration for all induction and challenge treatments.
The test substance group consisted of 20 animals. 10 animals were used as negative controls.
Induction: The animals were dermally treated with the test item three times at intervals of 7 (1^{st} to 2^{nd} induction) or 8 days (2^{nd} to 3^{rd} induction). The suitable areas of the body were shaved one day (24 h) before the first and second treatment and 30 min before the third treatment.
The inductions were performed with the 66.6 % test item paste. The animals in the test item group were treated with a patch loaded with the test item applied to the left flank and held in place on the skin with adhesive plaster.
Control group animals received a patch loaded only with the vehicle onto the left flank in each of the first to third inductions. The patches were removed after an exposure period of 6 h, and any remaining test item was removed with sterile physiological saline solution.
The volume applied per animal was 0.5 mL vehicle in the control group and 500 mg test item mixed with 0.25 mL vehicle in the test item group.
The treatment areas were assessed 24 h after patch removal. For this, the treatment areas were not shorn or chemically depilated.
Challenge: The challenge was performed 13 days after the last dermal induction.
For the challenge the backs and right flanks of the animals were shorn one day prior to the challenge. During the challenge a hypoallergenic patch loaded with the 66.6 % test item paste was applied and fixed to the right flank of each animal in the control and test item group. As a control a patch loaded only with the vehicle was applied and fixed also to the right flank, cranial to the test item patch. The patches were held securely in place on the skin with self-adhesive tape for 6 h.

The volume applied per animal was 0.5 mL vehicle and 500 mg test item mixed with 0.25 mL vehicle. At the end of the 6-hour exposure period, the patches were removed and the remaining test item was rinsed away with sterile physiological saline solution. 21 h later the skin of the animals was shorn in the region of the treatment sites. The skin reactions were evaluated 24 and 48 h after the end of the challenge exposure with the scoring system presented in OECD TG 406.

Results and discussions:

Table B.6.2-23: Results of the Buehler test (challenge)

	Test item group (20 animals)				**Control group (10 animals)**			
	Test item patch		Control patch		Test item patch		Control patch	
Hours after challenge exposure	24	48	24	48	24	48	24	48
No. of animals exhibiting skin reactions	0	0	0	0	0	0	0	0

There were no skin effects in the animal of the test item group and the control group during the three induction treatments. The challenge with the 66.6 % test item paste did not lead to skin effects in the animals of the test item group and in the control group.

Conclusion:

This Buehler Patch test for skin sensitisation in guinea pigs was run on the basis of OECD-Guideline no. 406 (adopted in July 17, 1992).
The study is considered supplementary.
The study design contains some weaknesses that limit the informative value.
First, dose-range-finding studies were performed in order to find the dose for sensitisation induction and challenge. OECD-Guideline no. 406 requires the highest dose to cause mild irritation for the induction exposure. For challenge exposure the highest non-irritating dose should be applied. A test item concentration of 66.6 % was chosen for the induction and challenge procedure even though no skin reaction was observed in the whole pilot study. This concentration does not include a mild irritation (for induction) and it is unclear whether this concentration matches the highest non-irritating dose (for challenge).Therefore, it remains questionable why the dose-range-finding study was not extended to higher concentrations above 66.6 % to investigate possible skin irritating effects at higher concentrations.
Another weakness of the study is the performance of the stability and homogeneity test. Although the test for skin sensitisation was conducted with a concentration of 66.6 %, both analyses were performed with 0, 1 and 40 % but not with 66.6 % of the test item. Neither a rationale for this study deviation nor the method of these analyses was given.
Occlusive conditions were neither claimed nor documented for the main study.
Another point is that the Buehler test conducted with three applications – compared with 9 applications – is no valid method for the evaluation of skin sensitisation (EFSA Handbook for the experts' meetings, Section 2: Mammalian toxicology, 2010).
Under the conditions used in the study and based on the information given in the report, beta-cyfluthrin did not induce any skin effect upon challenge in the test item group or in the control group (all scores 0). The reliability of the experimental technique was confirmed with alpha hexyl cinnamic aldehyde as positive control. 25 % or 45 % of the animals exhibited dermal reactions after the first or second challenge treatment, respectively.

Further studies available to RMS

The studies listed below were not submitted by the applicant for renewal of approval. However, the studies are available to the RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table 6.2-24). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table 6.2-24: Summary of skin sensitisation studies: cyfluthrin

Test	Concentrations	Vehicle	Result	Comment	Reference
Magnusson-Kligman (guinea pigs, male)	- Intraderm. ind.: 5 % - Topical Ind.: 50 % - Challenge: 50 % and 25 %	PEG 400	negative	- unclear why dose-range-finding study was not extended to higher concentrations (TG 406 1992)	[redacted] 1994 (23060) ASB2007-2854

A further study with cyfluthrin addressing skin sensitisation was submitted by the applicant [redacted], 1983, MO-01-004623; TOX9401875). The outcome of the study was negative. As no positive control (and no batch of the compound) is given, the study is considered not-acceptable.

For FCR 4545 no further studies were available.

B.6.2.7 Phototoxicity

Studies submitted with the dossier for the Renewal Assessment Report:

Data point: KCA 5.2.7 /01

Report: [redacted] 2013, ASB2014-7726
Beta-cyfluthrin: *In vitro* 3T3 NRU Phototoxicity Test.
Report No: 1556000 (October 2, 2013), M-481486-01-1; [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 432 (2004).

Deviations: The intensity of light was higher (2.25 - 2.85 mW/cm²) than suggested (1.5 - 1.9 mW/cm²) (recommended in OECD TG 2004).
The washing step with PBSS prior to the addition of Neutral Red solution was omitted (recommended in OECD TG 2004).
$2 \cdot 10^4$ cells per well were seeded instead of $1 \cdot 10^4$ cells per well (recommended in OECD TG 2004).
There is a lack of documentation (distance of the light source from the test system, no concentration-response curves) (demanded in OECD TG 2004).
Area of wavelengths chosen for emission not in absorption area of test compound (and further not optimal for positive control) (demanded in OECD TG 2004).

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is not acceptable.

(dates of exp. work: August 7 – August 14, 2013).

Materials and methods:

The phototoxic potential of beta-cyfluthrin (batch no.: PNBC0006963, purity: 99.1 %) was tested in an *in vitro* 3T3 NRU phototoxicity test in BALB/c 3T3 cells (isolated from mouse embryo, source: Dr. Liebsch, ZEBET, Berlin, Germany.).
A range-finding experiment was performed prior to the main experiment to define the test concentrations.
The following concentrations were tested in the range finding as well as in the main experiment (each concentration six times) with and without irradiation: 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63 and 31.25 µg/mL test item. The highest concentration was the highest technically feasible concentration, as higher concentrations were found to induce precipitation of the test item.
The negative control comprised cells treated with EBSS containing 1 % DMSO.
Chlorpromazine (CPZ) dissolved in EBSS was used as positive control. For chlorpromazine the following concentrations were applied: 6.25, 12.5, 25, 37.5, 50, 75, 100 and 200 µg/mL chlorpromazine without irradiation and 0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0 and 4.0 µg/mL chlorpromazine with irradiation.
About 24 h after seeding, the cells were incubated for 1 h with the respective concentration of the test item or control. Subsequently, the cells were either irradiated at 2.55 mW/cm^2 for 50 min or were stored for 50 min in the dark. Afterwards, the test or control solution was replaced by fresh culture medium and the cells were incubated for about one day.
For the determination of neutral red uptake to investigate the cytotoxic (without irradiation) and phototoxic (with irradiation) potential of the test item, the medium was then removed and neutral red-containing medium was added to each well. After 3 h to allow uptake of the vital dye into the lysosomes of viable cells the dye was extracted and its amount quantified photometrically at 540 nm. This absorbance shows a linear relationship with the number of surviving cells.

The results are evaluated according the validation study of Spielmann et al. (1998) 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.

Results and discussions:

The following criteria determined the acceptance of an assay:
After irradiation the cell viability of the solvent control is greater than 80 % of non-irradiated cells.
For the positive control CPZ the Photo Irritation Factor (PIF), defined as the ratio of toxicity, is greater than 6.
The mean OD_{540} of the solvent controls is greater than 0.4 (i.e. approx. twenty times the background solvent absorbance).
All acceptance criteria were met in the assay performed met the acceptance criteria (e.g. the mean of solvent control values of the irradiated group versus the non-irradiated group).

No cytotoxic effects were observed after treatment of cells with beta-cyfluthrin, neither in the presence nor in the absence of irradiation (Table B.6.2-25).
Therefore, neither ED_{50} nor the Photo-Irritancy-Factor (PIF) could be calculated. The resulting Mean Phototoxic Effect (MPE, obtained by comparing the response curves of treated cells in the presence and absence of irradiation) value was -0.037, hence, the test item is classified as not phototoxic.
The positive control chlorpromazine induced phototoxicity in the expected range after irradiation (Table B.6.2-26). The PIF value was 112.4, resulting in a MPE value of 0.717.

Table B.6.2-25: Optical density and viability of BALB/c 3T3 after treatment with beta-cyfluthrin

Irradiated				Non-irradiated			
Conc. [µg/mL]	OD_{540} Mean value	Standard Deviation	% of solvent control	Conc. [µg/mL]	OD_{540} Mean value	Standard Deviation	% of solvent control
Solvent control	0.8154*	0.0660	100	Solvent control	0.8584*	0.0479	100
0.24	0.8720	0.0543	107	0.24	0.8692	0.0527	101
0.49	0.8500	0.0282	104	0.49	0.8366	0.0357	97
0.98	0.8334	0.0367	102	0.98	0.8324	0.0423	97
1.95	0.8510	0.0693	104	1.95	0.8152	0.0225	95
3.91	0.7695	0.0400	94	3.91	0.8084	0.0330	94
7.81	0.7903	0.0224	97	7.81	0.7952	0.0376	93
15.63	0.7557	0.0292	93	15.63	0.7722	0.0266	90
31.25	0.7448	0.0208	91	31.25	0.7748	0.0243	90

* = mean OD_{540} out of 12 wells.

Table B.6.2-26: Optical density and viability of BALB/c 3T3 after treatment with chlor-promazine

Irradiated				Non-irradiated			
Conc. [µg/mL]	OD_{540} Mean value	Standard Deviation	% of solvent control	Conc. [µg/mL]	OD_{540} Mean value	Standard Deviation	% of solvent control
Solvent control	0.7766*	0.0411	100	Solvent control	0.9092*	0.0666	100
0.125	0.5782	0.0286	74	0.125	0.9146	0.0455	101
0.250	0.1631	0.0387	21	0.250	0.8015	0.0341	88
0.500	0.0817	0.0030	11	0.500	0.1920	0.0440	21
0.750	0.0906	0.0082	12	0.750	0.0947	0.0145	10
1.000	0.0984	0.0212	13	1.000	0.0552	0.0031	6
1.500	0.0804	0.0050	10	1.500	0.0524	0.0030	6
2.000	0.0858	0.0078	11	2.000	0.0524	0.0028	6
4.000	0.0895	0.0058	12	4.000	0.0533	0.0027	6

* = mean OD_{540} out of 12 wells.

Conclusion:

The phototoxicity test in BALB/c 3T3 cells complied with OECD-Guideline 432 (adopted in April 13, 2004).
The report submitted contains two deviations from the study plan: (1) The intensity of light was higher (2.25-2.85 mW/cm²) than recommended (1.5-1.9 mW/cm²) and (2) the washing step with PBSS prior to the addition of Neutral Red solution was omitted. However, these deviations are not assumed to have a detrimental impact on the results (acceptance criteria formulated in the guideline were met). A

further deviation from the recommendation included in the OECD-Guideline is the seeding of $2 \cdot 10^4$ cells per well instead of $1 \cdot 10^4$ cells per well.
Besides these practical imponderables, there is a lack of documentation in the study report (e.g. distance of the light source from the test system, no concentration-response curves).
According to OECD-Guideline 431 the applied light source must emit wavelengths that are absorbed by the test chemical in order to detect phototoxicity. However, the wavelength of the light source in the study was >320 nm even though the absorption of beta-cyfluthrin takes place at 200-300 nm (in 0.01 M phosphate buffer pH 7/acetonitrile; attached absorption spectrum at the end of the report) and the absorption peak of the used positive control chloropromazine (in ethanol) is obtained at a wavelength of 309 nm. It is unclear why the absorption spectra of the test compound and the positive control were not recorded in the solvent of the test system to avoid solvatochromic shifts.
In summary, the study cannot be accepted pending clarification of the impact of the selected wavelength of the light source on the results.
Under the conditions of the study and based on the information provided in the report, no phototoxic potency of the test compound is observed up to the highest achievable concentration (31.25 µg/ml test item in EBSS [1 % DMSO]). Since no cytotoxic effects were observed, only MPE values were determinable (-0,032 and -0,037). The PIF values and MPE values of the positive control chlorpromazine were within the expected range of 29.1 and 112.4 or 0.574 and 0.717, respectively.

The applicant submitted an expert statement on this issue that contains many reporting deficiencies (e.g. the cited literature is not submitted, the core beta-cyfluthrin phototoxicity study is not involved in the argumentation/reference list and the authors address is not given). It is stated that "*a new study will not solve the issue entcountered on the existing submitted study*". It is claimed that – even though a wavelength range >320 nm is given in the original study report – filtering in the UVB range would nevertheless leave enough "*UVB in the irradiation spectrum to excite chemicals typically absorbing in the UVB range*." However, this statement is not supported with experiments or literature. It is further argued that beta-cyfluthrin absorbs at a wavelength range (200-300 nm) where radiation-mediated cytotoxicity is observed in BALB/c 3T3 cells. It is further claimed that filtering UVB radiation would mimic the situation when light travels across the atmosphere. However, according to Commission regulation (EU) No. 283/2013 phototoxicity testing is required if the test compound absorbs electromagnetic radiation in the range 290-700 nm (molar extinction coefficient $\geq$ 10 L * mol^{-1} cm^{-1}). Beta-cyfluthrin consists mainly of isomer II and IV. The corresponding molar extinction coefficients are 80 $L \cdot mol^{-1} \cdot cm^{-1}$ and 85 $L \cdot mol^{-1} \cdot cm^{-1}$ at 291 nm (neutral conditions), respectively. Therefore, phototoxicity testing is needed. Furthermore, according to OECD TG 432 it is required that the "light source emits wavelengths absorbed by the test chemical". Taken this mechanistic requirement into account, the availbale phototoxicity study is considered not sufficient by the RMS to thoroughly assess the phototoxic potential of beta-cyfluthrin.

B.6.3 Short-term toxicity

No new data on short-term toxicity have been generated since Annex-I inclusion of cyfluthrin/beta-cyfluthrin and the publication of the addendum 1 (2002, ASB2014-9599). All studies have been previously submitted. For the renewal process they were again evaluated.
A literature research for the Renewal Assessment Report (RAR) including publications from the last 10 years was performed by the RMS. The publications were considered as supplemental information or where considered not acceptable.

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

B.6.3.1 Oral studies, rat

Data point:	KCA 5.3.1
Report:	[redacted], 1988, TOX9550271: FCR 4545 techn. - Subacute study of oral toxicity on rats. Report no.: 16384 (January 27, 1988); [redacted]
Guideline(s):	The test was run principally according to OECD-Guideline no. 407 which complies to Directive 92/69/EEC method B 7.
Deviations:	Reticulocytes, cholesterol, albumin, ornithine decarboxylase and gamma glutamyl transpeptidase, volume and specific gravity of the urine as well as food consumption were not determined. Additional investigations: Liver function (N-demethylase, O-demethylase, cytochrome P-450, alkaline phosphatase) and in a special histological manner the nerve tissue (brain, nervus ischiadicus, spinal cord, optic nerve), the muscle tissue and the eyes.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(Dates of exp. work: July - September 1985).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 98.5 %) was administered to Wistar rats (Bor:WISW (SPFCpb), source: [redacted]) once daily by gavage for 28 successive days in doses of 0, 0.25, 1, 4 and 16 mg/kg bw/d. The formulating agent was Cremophor/water, the applicated volume 10 ml/kg bw. The formulation was prepared fresh daily. A recovery period of 28 days without dosing followed to check the reversibility of toxic effects. The groups, consisting of 30 male and 30 female rats each, were divided into sub-groups with 5 males and 5 females each for:
- haematology, clinical chemistry and urinalysis at the end of dosing period or recovery period;
- gross pathology/histopathology at the end of dosing period or recovery period;
- special investigations of nervous system (perfusion fixation) and liver tissue at the end of dosing period and recovery period.

Statistical method: U-test of Wilcoxon, Mann, Whitney.

Results and discussions:

Mortality: 11 males and 12 females in the highest dose group (16 mg/kg bw/d) died.
Clinical signs: At 4 and 16 mg/kg bw/ the animals showed increased motility, digging and grooming

movements, salivation. At 16 mg/kg bw/d apathy, dyspnoe and athetotic and choreiform movements were additionally observed.
Body weight: At 16 mg/kg bw/d the males showed a retarded growth during the treatment period (week 1, 2: -7 %, week 3, 4: -10 %, -11 % of the body weight of control group). The body weight increased rapidly during the recovery period (week 5: -7 %, week 6 to 8: -4 % of the weight of control group).
Haematology, clinical chemistry (serum, liver tissue), urinalysis: All values were within the normal range.
Gross pathology, organ weights: In all groups nearly the same pre-terminal or post mortem spontaneous findings were noted as in the investigations of acute toxicity (e.g. mottled, pale or red organs, sometimes distended). These findings were not connected with the test substance.
The absolute and relative liver weights were significantly elevated in females at 4 mg/kg bw and above. In males the relative liver weight was slightly increased in the 16 mg/kg bw/d group. It could not be ruled out that this finding was caused by the substance although the increase in females was not dose related (see next table).

Table B.6.3-1: Absolute and relative liver weight at the end of dosing period

Dose (mg/kg bw) **Sex**	**0** **M/F**	**0.25** **M/F**	**1** **M/F**	**4** **M/F**	**16** **M/F**
Liver absolute (mg)	10316/ 6287	10264/ 6604	9897/ 6801	9677/ 7375**	9943/ 7024*
Liver, relative (mg/100 g bw)	3998/ 3525	3926/ 3719	3989/ 3796*	3905/ 4044**	4244*/ 3994*

* = p <0.05; ** = p <0.01.

Histopathology: There were no test substance related findings. This concerned also the nerve tissues investigated in detail (brain, nervous ischiadicus, spinal cord, muscle, eyes including optic nerve).
Reversibility: Alterations which were noted at the end of dosing period were not apparent at the end of the recovery period.

Conclusion:

The doses of 0.25 and 1 mg/kg bw/d beta-cyfluthrin were tolerated without adverse toxic effects. The noticed alterations after the dosing period were reversible. The no observed adverse effect level (NOAEL) of 1 mg/kg bw/d was based on the slight increase in liver weight which occurred from this dose onward in the female animals.

Re-evaluation by the RMS (2015):

16 mg/kg bw/d: Mortality (11 males, 12 females); clinical signs (increased motility, digging and grooming movements, salivation, apathy, dyspnoe, athetotic and choreiform movements), males showed a retarded growth during the treatment period; increased absolute and relative liver weights in females, slightly increased relative liver weight in males.
4 mg/kg bw/d: clinical signs (increased motility, digging and grooming movements, salivation); increased absolute and relative liver weights in females.
The doses of 0.25 and 1 mg/kg bw/d beta-cyfluthrin were tolerated without adverse toxic effects. The slight but significant increase in relative liver weight in female animals at 1 mg/kg bw/d was not considered an adverse change since absolute liver weights were not affected, no changes in biochemical parameters and no histopathological correlates were found.
NOAEL: 1 mg/kg bw/d.
All effects were reversible during the recovery period.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.3.2
Report:	[redacted], 1988, TOX9550272: FCR 4545 - Subchronic toxicological study on rats (administration with feed for 13 weeks). Report no.: 16807 (June 21, 1988) and 16807A (Addendum; Bomhard, E., Rühl-Fehlert, C., June 21, 1994); [redacted]
Guideline(s):	The test was run principally according to OECD-Guideline no. 408 which complies to Directive 87/302/EEC part B.
Deviations:	Only 15 animals/dose group were used, functional observations were not conducted, ornithine decarboxylase and gamma glutamyl transpeptidase were not determined. In addition liver function (N-demethylase, O-demethylase, cytochrome P-450, triglycerides, alkaline phosphatase), fluoride content in bones and teeth were determined. Additional measurements: Hematology, clinical chemistry and urinalysis after 4 weeks, ophthalmology in week 1.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(dates of exp. work: July - November 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16001/85, purity: 99.7 %) was administered to 15 Wistar rats (Bor:WISW (SPFCpb), source: [redacted]) per sex and dose for 90 successive days via the food. The nominal doses were 0, 30, 125 and 500 ppm. The doses were selected on the base of a preliminary range finding experiment. The concentration in the diet was checked at regular intervals. One additional control group and one recovery group of 15 animals per sex each were included to check the reversibility of toxic effects. The recovery group was dosed with 500 ppm beta-cyfluthrin for 90 days and remained without dosing for 28 days (follow-up period). The nominal doses corresponded to a test substance intake of 2.3, 9.5, 38.9 and 37* mg/kg bw/d for males and 2.5, 10.9, 42.4 and 43* mg/kg bw/d for females (*recovery group).
Statistical method: U-test of Wilcoxon, Mann, Whitney.

Results and discussions:

Mortality: A relationship between the death of 2 animals at 500 ppm and the test substance intake could not be completely ruled out.
Clinical signs: At 500 ppm: A poor general health condition and an uncoordinated gait were observed during the first week of treatment. The necrosis and sores in the head and neck region of 6 males and 3 females were attributed to the increased preening movements which led to skin injuries. No ophthalmological findings were observed.
Body weight, food and water intake: At 500 ppm during the first two weeks a drastically reduced body weight gain was noted (approx. -30 % of the weight of control group). During the further course of the treatment with beta-cyfluthrin the weight gain was decreased in males and only slightly decreased in females (weight of control group from week 3 to week 13: from -20 % to -10 % in males, from -11 % to -6 % in females). At the end of treatment the final body weight was decreased in males and females of the 500 ppm group (approximately 10 % less than control group). The food intake was similar in all groups. The water consumption of the animals of the 500 ppm group was markedly reduced compared to that of the control group.
Haematology, clinical chemistry (serum, liver tissue), urinalysis: At 500 ppm a significant decrease in erythrocyte count, haemoglobin level and haematocrit (5-13 % of control values) was noted in males and females after 4 weeks and during the recovery period. In the urine of animals of the high dose group (500 ppm) increased calcium levels were noted after 4 week and during the recovery period.

This change was not noted after 3 months of treatment.
Gross pathology, organ weights, fluoride content, histopathology: No substance related findings were noted.

Addendum to Report no. 16807 (Bomhard and Rühl-Fehlert, 1994, TOX9550272):
This addendum contains information not included in the original study report no. 16087. These are: analytical results on the batch no. of the test compound used and results from additional histopathological investigations of oesophagus, parathyroids and female mammary gland. In addition, locations of sections of brain and spinal cord investigated previously are specified.

Conclusion:

There were no gross pathological findings in the organs investigated. The histopathological examination did not reveal any test substance-related changes at concentrations up to 500 ppm.

Re-evaluation by the RMS (2015):

500 ppm (38.9 and 37* mg/kg bw/d for males and 42.4 and 43* mg/kg bw/d for females (*recovery group): Mortality (2 males); Clinical signs: poor general health condition, uncoordinated gait during the first week of treatment; necrosis and sores in the head and neck region of 6 males and 3 females; reduced body weight gain during the first two weeks (approx. -30 % of the weight of control group) during the further course of the treatment (from -20 % to -10 % in males, from -11 % to -6 % in females) and at the end of treatment (approx. 10 % less than control group); decreased water intake; significant decrease in erythrocyte count, haemoglobin level and haematocrit (5-13 % of control values) in males and females after 4 weeks and during the recovery period; increased calcium levels in the urine after 4 weeks and during the recovery period.
NOAEL: 125 ppm (9.5 and 10.9 mg/kg bw/d for male and female rats)
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.3.2
Report:	[redacted]. TOX9401881 Three-month Subacute Toxicity study of FCR 1272 in Rats - Report no.: 264 (July 31, 1983); [redacted]
Guideline(s):	Testing guideline of MAFF, Japan (47 Nosei, no. 2538, June 14, 1972) and EPA Guideline (Proposed Guidelines for registering Pesticides in the U.S., Federal Register, vol. 43. no. 163, August 22, 1978). Partly OECD 408 (1998)
Deviations:	Blood clotting potential/time, electrolytes, total bilirubin, γ-glutamyl transpeptidase, urea, and albumin were not determined in blood. Ophthalmologic examinations were not performed. Uterus and thymus weight were not taken. Histopathology was not performed for brain, pituitary, salivary glands, large intestine, liver, kidney, adrenal glands. Spleen, heart, lung, mammary gland, prostate, and seminal vesicles.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Acceptable

(Dates of exp. work: July - October 1982)

Materials and methods:

Groups of 28 male and 28 female Sprague-Dawley rats (CF-1, source: Charles River Japan Inc.) received cyfluthrin (batch no.: 816170019, purity 95 %, a maximal concentration of clay of 0.4 % in 1000 ppm prepared feed) for 3 months via the feed in concentrations of 0, 100, 300, and 1000 ppm corresponding to: 6.21, 18.98 or 60.90 mg/kg bw/d in males, and 7.29, 21.22 or 68.47 mg/kg bw/d in females. Eight rats per sex from each group were observed for a further month after the end of the treatment.
Statistics: The significance of intergroup differences was checked using Student's t-test. Data on differential leukocyte counts were analysed after reverse sinusoidal transformation. Data on blood biochemical tests and organ weights were analysed after using Smirnoff's rejection test.

Results and discussions:

The animals of the 1000 ppm group exhibited a slightly straddle-legged gait and salivation in the first half of the treatment period. No signs were recorded towards the end of the treatment or during the recovery period.
At 1000 ppm both sexes showed reduced food consumption and a depressed body weight gain.

Table B.6.3-2: Body weight

Dose [ppm] Sex	**0 M/F**	**100 M/F**	**300 M/F**	**1000 M/F**
Body weight (3 mo) [g] ± SD [g] Signi #	429/239 32/23	425/240 43/19	420/234 36/18	378/213 39/22 **/**

[# = * p<0.05/** p<0.01]

No influence on the haematological or urine analytical parameters was noted.
Of the clinicochemical parameters studied glucose levels were reduced (in male rats on 300 and 1000 ppm and in females on 1000 ppm). The effect was reversible. Blood urea nitrogen (BUN) was significantly elevated in males receiving 300 ppm and above and in females receiving 1000 ppm. Males in the 1000 ppm group displayed a significant elevation of serum ASAT.

Table B.6.3-3: Clinical laboratory tests

Dose [ppm] Sex	**0 M/F**	**100 M/F**	**300 M/F**	**1000 M/F**
Glucose (3 mo) [mg/dl] ± SD [mg/dl] Signi #	112/101 18/12	106/99 17/15	95/97 18/13 **/ .	79/84 8/10 **/**
ASAT (3 mo) [mu/ml] ± SD [mu/ml] Signi #	66.6/72.1 9.8/14.1	70.5/63.4 13.0/10.2 /*	67.3/58.8 11.9/14.6 /**	77.4/67.0 9.2/13.6 **/ .
BUN (3 mo) [mg/dl] ± SD [mg/dl] Signi #	18.5/20.4 2.1/2.3	19.2/20.4 2.3/1.6	20.2/19.7 1.9/2.9 */ .	21.4/23.7 2.4/3.6 **/**

[# = * p<0.05/** p<0.01];

The results of the necropsies and the organ weight determinations were not suggestive of any effects attributable to the treatment. The significant changes in absolute and relative organ weights were related to the body weight decrease at termination.
Histopathological analysis revealed slight axonal degeneration of individual sciatic nerve fibres in 5 out of 20 males and 3 out of 20 females on 1000 ppm. Examination at the end of the follow-up period revealed similar alterations in 1 out of 8 males in the 1000 ppm group. These results suggested that morphologic change of sciatic nerve seen in animals receiving cyfluthrin was not progressive, and found gradually repairable following withdrawal of the compound.

Conclusion:

The NOEL of 100 ppm, corresponding to 6.21 mg/kg bw/d in male rats and of 300 ppm, corresponding to 21.22 mg/kg bw/d in female rats was based on transitory reductions in blood glucose levels of male rats on 300 ppm and of female rats on 1000 ppm. Furthermore, abnormal gait, salivation and morphological changes in nerve fibres were observed at the highest dose level. All changes were repairable following withdrawal of the compound.

Re-evaluation by the RMS (2015):

NOAEL: 100 ppm (6.21 mg/kg bw/d).
Although a variety of parameters were not examined, the study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of cyfluthrin (1996, ASB2010-10436), the study was considered acceptable.

B.6.3.2 Oral studies, dog

Data point:	KCA 5.3.2
Report:	[redacted], 1987, TOX9550274: FCR 4545 - Study of subchronic oral toxicity to dogs (13-week feeding study). Report no. 16180 (November 04, 1987); [redacted]
Guideline(s):	The test was run principally according to OECD-Guideline no. 409 which complies to Directive 87/302/EEC part B.
Deviations:	Ornithine decarboxylase and gamma glutamyl transpeptidase were not determined. In addition were determined: Body temperature, pulse rate, liver function (N-demethylase, cytochrome P-450, triglycerides), fluoride content in bones and teeth.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(dates of exp. work: March - June 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16001/85, purity: 99.7 %) was administered to 4 beagle dogs (Bor:BEAG), source: [redacted]) per sex and dose for 90 successive days. The animals received the substance via the food in doses of 0, 10, 60 and 360 ppm (group I, II, III, IV). The doses were selected on the base of a preliminary range finding experiment. The concentration in the diet was checked at regular intervals. The doses in food corresponded to a test substance intake of 3.68, 22.1, 131 mg/animal/d for both males and females.
Statistical method: Calculation of arithmetic means and standard deviation.

Results and discussions:

No mortality occurred.
At all doses no reflex changes (pupillary reflex, corneal reflex, patellar tendon reflex, bending and righting reflex), no ophthalmological findings and no alteration of body temperature and pulse rate were observed.
Clinical signs: At 360 ppm the four dogs showed motor disturbances principally in the region of hind limbs (uncertain, staggering gait, buckling), sporadically vomiting and diarrhoea. The motor disturbances did not occur every day, persisted on most occasions for 6-8 hours after feeding and then disappeared until the next feeding.

Body weight, food and water intake: In group IV the weight gain was decreased in females (+ 0.4 kg) in comparison both to the control females (+ 1.0 kg) and to the females of group II (+ 1.6 kg) and III (+ 1.2 kg). There were no differences between the groups in respect to food intake and water consumption.
Haematology, clinical chemistry (serum, liver tissue), urinalysis: All values were within the normal range.
Gross pathology, organ weights, fluoride content in bones and teeth, histopathology: No substance related findings were noted.

Conclusion:

The NOEL of 60 ppm, corresponding to 1.5 mg/kg bw/d, was based on motor disturbances and a reduced body weight gain in females at the dose of 360 ppm.

Re-evaluation by the RMS (2015):

The NOAEL of 60 ppm, corresponding to 2.4 mg/kg bw/d, was based on motor disturbances, vomiting and diarrhoea in three male and one female dogs, and a reduced body weight gain in females at the next higher dose of 360 ppm.
The applicant proposed to revise the NOAEL derived for this endpoint. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) (Section B.5.3.1.3) the NOEL of 60 ppm from the 90-day feeding study in dogs has been converted into a dose of 1.5 mg/kg bw/day using a default conversion factor. However, actual food consumption data are available for this study (also reported in the monograph). It is preferable to calculate the doses of the NOEL and LOEL based on actual consumption data. Hence, the revised NOEL is 2.4 mg/kg bw/day.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.5
Report:	[redacted], 1983, TOX9401903: FCR 1272 - Chronic toxicity to dogs on oral administration. Report no.: 11983 (August 3, 1983); [redacted]
Guideline(s):	The test was run principally according to OECD-Guideline no. 452/409 which complies to Directive 87/302/EEC part B.
Deviations:	The purity of the test substance (cyfluthrin) was not given. Ornithine decarboxylase and gamma glutamyl transpeptidase were not determined. Histopathology: Trachea was not investigated. The intestine was studied as one organ, not its 6 constituent parts separately. In addition were determined: Body temperature, pulse rate.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Not acceptable

(Dates of exp. work: January 1982 - January 1983).

Materials and methods:

Groups of 6 male and 6 female pure-bred beagle dogs (source: [redacted]) received cyfluthrin (mixed batch composed of five different batches, in the form of a 50 % pre-mix with colloidal silicic acid (Wessalon S) via the dry feed for 12 months in concentrations of 0, 40, 160, and 640 ppm, equivalent to approx. 0, 1, 4 or 16 mg/kg bw/d.
Statistical method: Calculation of arithmetic means and standard deviation. Analysis of significance was conducted according to U-test of Wilcoxon, Mann, Whitney.

Results and discussions:

No mortality occurred.
At all doses no reflex changes (pupillary reflex, corneal reflex, patellar tendon reflex, stretch, bending and righting reflex), no ophthalmological findings and no alteration of body temperature and pulse rate were observed.
Two of the animals on the highest dose (640 ppm) each exhibited on a single occasion slight disturbances of movement chiefly affecting the hind legs. However, the gross pathological and histopathological examinations of the nervous system did not reveal deviations from the physiological norm in these, like in all other, animals.
Animals in the 640 ppm group also exhibited an increased incidence of vomiting and soft to watery faeces throughout the entire period of study. No major differences were found between the groups in terms of food and water consumption. The males on 640 ppm put on less weight than those in the other groups.

Table B.6.3-4: Test compound intake and body weight gain

Dose [ppm] Sex	0 M/F	40 M/F	160 M/F	640 M/F
Test compound intake [mg/animal/wk]*	0/0	109/106	443/418	1682/1756
Body weight (wk -1) Means [kg]**	8.6/8.4	8.6/8.4	8.5/8.2	8.5/8.2
Body weight (wk 52) Means [kg]**	12.3/11.8	12.8/11.6	13.3/11.8	11.1/12.0
Body weight gain Means [kg]**	3.7/3.4	4.2/3.4	4.8/3.6	2.6/3.8

* The calculated substance intake in [mg/kg bw/d] was only given in tier I, but not in the original report.
** No statistical evaluation was performed for males and females, separately.

No laboratory, morphological, gravimetric or histopathological findings that could be interpreted as evidence of somatic or harmful effects were recorded.

Conclusion:

The NOEL of 160 ppm, equal to approximately 4 mg/bw kg/d, was based on increased incidence of vomiting and soft faeces, on reduction in body weight gain and impaired motility at the dose of 640 ppm.

Re-evaluation by the RMS (2015):

The notifier proposed to revise the NOAEL derived for this endpoint because actual test substance intake data are available for this study. The proposed revised NOAEL is 6.2 mg/kg bw/day (instead of 4 mg/kg bw/d) and the revised LOAEL is 25 mg/kg bw/day (instead of 16 mg/kg bw/d) based on reduction in body weight gain, impaired motility (slow and unsure movements, clumsy gait in the hind quarters) in two male dogs, vomiting and soft faeces at the dose of 640 ppm.
The study is now considered to be not acceptable since no information on the purity of the test substance is given. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered to be acceptable.
The study is superseded by a guideline GLP study (see studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599): Jones R.D., Hastings T.F. 1997, TOX9800225).

B.6.3.3 Dermal study, rat

Data point:	KCA 5.3
Report:	El-Elaimy, 1986, TOX9401884: Biochemical disturbance in liver function and whole blood AchE due to repeated dermal application of Baythroid to rat. - Proc. Zool. Soc. A. R. Egypt. 10, 51-60, 1986.
Guideline(s):	No guideline-conform study.
Deviations:	No study protocol was presented, batch-no. and purity were not provided for the test material, only a summary description of the results and summary tables without single data were given, the daily dosage levels were not clearly specified, the calculation of the dosage was not clearly specified (calculation as mg/kg bw can be assumed).
GLP:	No
Acceptability:	Not acceptable

Materials and methods:

Groups of 5 male albino rats (strain: Rattus norvegicus, purchased from the Egyptian Organisation for Biological and Vaccine Production) were treated for 7 successive days. According to the original report, each rat in tested groups received a daily dermal dose of 0, 200, 400, 600, and 800 µl of a 40 % formulation of cyfluthrin dissolved in ethyl alcohol containing approx. 0, 80, 160, 240, and 320 mg of the active material. Exposure was performed to the shaven dorsal skin under occlusive conditions for 24 h a day. Each interval of 24 hours the pads were removed and the tested concentration was applied, each time without removing the residues of the proceeding applications.
The surviving animals were sacrificed 24 hours after the final treatment.
A further group of 20 male albino rats was treated with 250 µl (corresponding to 100 mg cyfluthrin) for 7 days. Five animals each were investigated at intervals of 5, 10, 15 and 20 days after the end of the treatment.
The examinations comprised liver function tests (measurements of serum alanine (GOT) and aspartate aminotransferases (GPT), serum alkaline phosphatase, total proteins, albumin, globulins, bilirubin, whole-blood acetylcholinesterase activities).
Statistical method: Student's t-test.

Results and discussions:

600 and 800 µl (240 and 320 mg): Chewing, licking, salivation, motor symptoms (pawing, burrowing, tremor, slow twisting, clonic seizures), choreoathetosis. The animals in the two highest-dose groups died within 4 days of the start of the treatment.
200 and 400 µl (80 and 160 mg):
Significant and dose-related increases in alkaline phosphatase, GOT and GPT activities, and total bilirubin. Acetylcholinesterase (AChe) activities, total protein, total globulin, albumin concentrations and the A/G ratio were significantly and dose-related reduced.
These parameters can be interpreted as signs of an impairment of liver function.
The effects were reversible within 20 days after dermal application of 250 µl/kg.

Conclusion:

The LOEL of 80 mg/kg bw was based on the increase in serum transaminases and the inhibition of AchE in animals treated with 80 and 160 mg/kg bw.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable since no study protocol was presented, no information on the batch and purity of the test substance, and no individual data are given. In the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered to be supplemental.

B.6.3.4 Dermal studies, rabbit

Data point:	KCA 5.3.3
Report:	[redacted], 1980, TOX9401883: FCR 1272 - Subacute dermal toxicity study on rabbits. Report no.: 8928 (February 05, 1980); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compulsory. The method used complied, however, to a great extent to then in force EPA Guidelines (Proposed Guidelines for Registering Pesticides in the U.S., Federal Register, Vol. 43, no. 163, August 22, 1978)
Deviations:	Only two treated groups were used. Clinical chemistry examinations did not comprise calcium, phosphorus, chloride, potassium, albumin, bilirubin and total serum protein measurements.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Acceptable

(Dates of exp. work: August 1979).

Materials and methods:

Groups of 6 male and 6 female albino rabbits ([redacted]) were treated with cyfluthrin (batch no.: 16001/79, purity: 83.5 %, formulated in polyethylene glycol 400) for 3 weeks in doses of 0, 50, and 250 mg/kg bw (6 hours/day, 5 days/week).
The test solutions were prepared every day just before application. They were adjusted every week according to the most recent body weight measurements. The backs and flanks of the rabbits were clipped free 48 hours prior to treatment. On three males and females of each group the skin was additionally abraded 24 hours prior to treatment. New growth of hair in and around the treated skin area was removed twice weekly. The treated skin area (approx. 6 x 7 cm) was left uncovered. At the end of each contact time, the treated skin was washed with acetone (only after 1st administration) or 94 % ethyl alcohol and then with soap and water.

Results and discussions:

No mortalities occurred. FCR 1272 did not cause any skin irritations or other damage to intact or abraded skin. Physical appearance, behavioural patterns, body weights, haematological data, clinical chemical data, urinalyses, organ weight measurements, gross pathology and histopathology did not provide any indication of alterations due to treatment with the test compound.

Conclusion:

There were no indications of FCR 1272 having a systemic effect on the experimental animals. The NOEL of 250 mg/kg bw was based on the absence of effects in the highest dose group.

Re-evaluation by the RMS (2015):

The NOAEL of 250 mg/kg bw was based on the absence of systemic or local skin effects in the highest dose group.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

B.6.3.5 Inhalation studies, rat

Data point:	KCA 5.3.3
Report:	[redacted], 1988, TOX9550275: FCR 4545 (common name: Cyfluthrin K+L, suggested) - Study of the range-finding subacute inhalation toxicity to rats in accordance with OECD-Guideline no. 403. Report no.: 16593 (April 7, 1988); [redacted]
Guideline(s):	The technical standard was based on OECD-Guideline no. 403 which complies to Directive 92/69/EEC method B 2.
Deviations:	Exposure at 6 h/d for 5 consecutive days instead of 1 x 4 hours, additional recording of body weight on day 4 and recording of reflexes (cornea, pinna, light, myotactic, startle, righting reflex).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(dates of exp. work: September - October 1987).

Materials and methods:

Groups of 10 male and 10 female Wistar rats (BOR:WISW (SPFCpb), source: [redacted]) received beta-cyfluthrin (batch no: 16001/87, purity: 98 %) via inhalation (head-nose only). The substance was administered as an aerosol (dynamic spraying) at 6 h/d for 5 consecutive days. The formulating agent was ethanol/PEG 400 (1:1). The nominal concentrations were 0 (vehicle, group I), 2, 20 and 200 mg/m³ air (group II, III, IV) corresponding to analytical concentrations of 0, 0.25, 3.8 and 28 mg/m³ air.
Statistical methods: For different parameters different methods (U-test of Wilcoxon, Mann, Whitney, modified by Walter, Chi-square-test, Fisher-test, special tests for characterisation of the aerosol distribution).

Results and discussions:

No mortalities occurred.
Analysis of test atmosphere: Inhalable particle content 100 % (particles <5 µm).
Clinical signs (including reflexes):
In Group III and IV (3.8 and 28 mg/m³ air):
Starting after the 3rd exposure (day 2), the rats of group III showed an unkempt fur and piloerection. The signs disappeared until to the next exposure. After each exposure the rats of group IV showed an unkempt fur, piloerection and a reduced activity more pronounced and lasting longer but without indications of a progressive severity. No reflex changes were seen.
Body weight: After the 5th exposure (day 4) in group III the body weight was marginally lowered in comparison to the control group (males 4 %, females 3 %), but not thereafter. At the same day in group IV a significant effect was seen (males and females 7 %), which was only marginal two days later.
Gross pathology: Lung changes (″hepatoid foci″) were observed in group III (4/20) and group IV

(6/20).

Conclusion:

The NOEL was 0.25 mg/m³ air based on clinical signs as the most sensitive parameter as well as on a transient effect on the body weight and on the lung findings at higher doses.

Re-evaluation by the RMS (2015):

The NOAEL was 0.25 mg/m³ air based on clinical signs, on a transient effect on the body weight and on the lung findings at higher doses.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.3.3
Report:	[redacted], 1989, TOX9550276: [redacted] FCR 4545 (c. n.: Betacyfluthrin, proposed) - Subacute inhalation toxicity study in the rat according to OECD-Guideline no. 412. Report no.: 18146 (June 28, 1989); [redacted]
Guideline(s):	The test was run according to OECD-Guideline no. 412 which complies to Directive 92/69/EEC method B 8.
Deviations:	Ornithine decarboxylase and gamma glutamyl transpeptidase were not determined. Additional investigations: Liver function (N-demethylase, O-demethylase, cytochrome P-450, triglycerides), recording of reflexes (cornea, pinna, light, myotactic, startle, righting reflex, weekly), lung function tests.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(dates of exp. work: February - March 1989).

Materials and methods:

Groups of 10 male and 10 female Wistar rats (BOR:WISW (SPFCpb), source: [redacted]) received beta-cyfluthrin (batch no: 16001/87, purity: 97.9 %) via inhalation (head-nose only). The substance was administered as an aerosol (dynamic spraying) during 6 hours daily, five times per week for 4 weeks. The formulating agent was ethanol/PEG 400 (1:1). The nominal concentrations were 0 (air (group I), vehicle (group II)), 1.5, 15 and 150 mg/m³ air (group III, IV, V) corresponding to analytical concentrations of 0.2, 2.7 and 23.5 mg/m³ air. If a minute volume of 1 L per kg for rats is used as an approximate basis, these doses correspond to a substance intake of 0.07, 0.9 and 8 mg/kg bw/d.
Lung function tests (in the last third of study): 5 anaesthetised male rats per group were placed in a plethysmograph for a minimum of 2 hours. Several lung function parameters (e.g. respiration rate, respiratory minute volume, expiratory flow (peak, mean), tidal volume, inspiratory time, expiratory time) were evaluated. Other measurements: Acetylcholine provocation test, test for CO diffusion capacity.
Statistical methods: For different parameters different methods (U-test of Mann, Whitney, modified by Walter, Chi-square-test, Fisher-test, Variance analysis (ANOVA), Box's test, Tukey-Kramer-test, modified by Games and Howell, special tests for characterisation of the aerosol distribution).

Results and discussions:

Analysis of test atmosphere: Inhalable particle content up to 99.9 % (particles <5 µm).
No mortalities occurred.
Clinical signs (including reflexes): From the 1st to the last day, directly after the end of exposure, the rats of group V (150 / 23.5 mg/m³ air) showed an unkempt fur, piloerection, sometimes a slightly reduced motility but mainly an increased activity. No indications of a progressive severity and no sex specific differences occurred. No reflex changes and ophthalmological findings were seen.
Body weight: There was a statistically significant influence on body weight and body weight gain from 15 / 2.7 mg/m³ air onward (Table B.6.3-6).
Hematology, clinical chemistry (serum, liver tissue), urinalysis: In the females of group V, the leucocytes and the lymphocyte subpopulation were slightly reduced. Also in females of this group, an increase of the alkaline phosphatase activity as well as a lowering of the cholesterol, protein, potassium, calcium and phosphate concentration were considered to be induced by beta-cyfluthrin (Table B.6.3-5). In males, a slight acidification of the urine was noted at 2.7 mg/m³ air and above. The slightly changed clinical chemical parameters and the lowered urine pH were interpreted as a result of compensatory reactions due to a slight respiratory acidosis and not as an effect of a specific organ dysfunction. The investigation of liver tissue produced no evidence of toxicologically relevant changes.
Lung function tests: The lung function tests produced no evidence of pathophysiologically significant changes in the lung mechanisms or diffusion capacity. The decreased respiratory rate from 2.7 mg/m³ air onward was considered to be due to a reflex bradypnoe. In the acetylcholine provocation test no increased readiness of the bronchial tract to react to non-specific stimuli was observed.
Ophthalmological examinations: The ophthalmological examinations revealed no substance-induced changes.
Gross pathology, organ weights: There were no indications of a test substance induced grossly apparent organ damage. The absolute and relative weights of thymus and spleen were reduced at the highest dose level mainly (Table B.6.3-6).
Histopathology: There were no test substance related findings.

Table B.6.3-5: Haematological and clinical chemical parameters (week 4)

AC (mg/m³ air) **Sex**	**0 a** **M/F**	**0 v** **M/F**	**0.2** **M/F**	**2.7** **M/F**	**23.5** **M/F**
Lymphocytes (%)	85.5/ 85.4	85.5/ 87.7	86.3/ 88.1	88.5/ 85.5	83.2/ 79.7**
Leucocytes (10E9/L)	5.5/ 4.0	5.8/ 3.8	5.1/ 4.6	4.9/ 4.8	4.3/ 2.8
Alkaline phosphatase (U/L)	368/ 230	344/ 224	367/ 225	324/ 244	352/ 279**
Protein (g/L)	63.8**# 63.3	60.8/ 64.1	59.8/ 62.8	59.7/ 63.4	59.8/ 60.8**
Cholesterol (mmol/L)	2.11*/ 2.03	1.83/ 1.89	1.70/ 1.50**	1.97/ 1.68	1.77/ 1.26**
Potassium (mmol/L)	5.6/ 5.0	5.3/ 5.6	5.4/ 5.6	5.2/ 5.8	5.0/ 4.6**
Calcium (mmol/L)	2.70*/ 2.56	2.61/ 2.59	2.57/ 2.54*	2.61/ 2.60	2.60/ 2.49**
Phosphate (mmol/L)	3.02/ 2.17*	2.75/ 2.59	2.60/ 2.66	2.88/ 2.55	2.57/ 2.09**

AC = Analytical concentrations; a = air control ; v = vehicle control; * = p <0.05, ** = p <0.01; # = significant against other control.

Table B.6.3-6: Body weight, spleen and thymus weights

AC (mg/m³ air) Sex	0 a M/F	0 v M/F	0.2 M/F	2.7 M/F	23.5 M/F
Body weight (g), day 0	185/ 171	182/ 176	184/ 174	181/ 171*	183/ 174
Body weight (g), week 4	245*#/ 184	236/ 191	227/ 186	219*/ 178**	211**/ 179**
Spleen, absolute (mg)	472/ 406	452/ 386	391**/ 407	411/ 365	343**/ 308**
Spleen, relative (mg/100 g bw)	192/ 214	192/ 200	173/ 215	188/ 205	160**/ 168**
Thymus, absolute (mg)	330/ 240	275/ 247	234*/ 229	271/ 230	177**/ 157**
Thymus, relative (mg/100 g bw)	134/ 127	116/ 128	102/ 121	122/ 129	83**/ 85**

AC = Analytical concentrations; a = air control ; v = vehicle control; * = p <0.05, ** = p <0.01; # = significant against other control.

Conclusion:

On the basis of the most sensitive parameter (reduced body weight gain), the NOEL in this study was 0.2 mg beta-cyfluthrin/m³ air. This dose corresponds to a daily substance intake of approx. 0.07 mg/kg bw/d.

Re-evaluation by the RMS (2015):

At 23.5 mg/m³ air: clinical signs (unkempt fur, piloerection, sometimes a slightly reduced motility but mainly an increased activity.) in males and females, in females slightly reduced leucocytes and lymphocytes, increase of the alkaline phosphatase activity, lower cholesterol, protein, potassium, calcium and phosphate concentration.
Lower body weight gain in animals of either sex, slight acidification of the urine in males from 2.7 mg/m³ air onward.
The NOAEL is considered to be 0.2 mg/m³ air (0.07 mg/kg bw/d).
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point: KCA 5.3.3

Report: [redacted], 1984, TOX9401887:
FCR 1272 - Study for subchronic inhalative toxicity to the rat for 13 weeks (exposure 63 x 6 hours) - Report no.: 12436 (February 1, 1984 report), 15469 (January 22, 1987 addendum), 15469A (July 30, 1987 addendum); [redacted]

Guideline(s): The test was run according to OECD-Guideline no. 413 which complies to Directive 87/302/EEC, part B.

Deviations: Main study: Food and water consumption, clotting potential, chloride, potassium, sodium, calcium, phosphate, cholesterol, triglycerides, albumin, total protein, globulin, creatinine levels were not determined; histopathology was not performed for bone marrow, femur, mammary gland, pituitary, prostate, seminal vesicles, spinal cord, sternum, thymus. Ophthalmologic examination was not performed. In addition liver enzymes were determined (N-demethylase, O-demethylase, cytochrome P-450)

GLP: When the study was performed, GLP was not compulsory.

Acceptability: Acceptable

(Dates of exp. work: April - July 1983).

Materials and methods:

Groups of 10 male and 10 female Wistar rats (Bor:WISW (SPFCpb), source: [redacted]) were exposed to cyfluthrin (batch no: 816170019, purity: 94.9 % formulated in polyethylene glycol E 400 : ethanol (1 : 1)) in aerosol form for 12 weeks (5 times/week; 63 x 6 h) under dynamic conditions in mean analytical concentrations of 0, 0.09, 0.71, and 4.52 mg/m³ air (nominal concentration: 0, 0.5, 3.0, 20.0 mg/m³ air) (head-nose exposure).
Statistical method: Means, standard deviation, confidence intervals. The values of the control groups were compared to the dosage groups by the U-test of Mann-Whitney-Wilcoxon.

Results and discussions:

Analysis of the test atmosphere: Stable and reproducible conditions of exposure were achieved. The aerosol had a mean mass media aerodynamic diameter (MMAD) of about 2.9 mm. Over 85 % of the particle mass was therefore respirable (particles <5 µm).
No mortalities occurred.
Clinical signs:
Non-specific behavioural disturbances with agitation (erected tail) were observed in all animals (both sexes) of the highest dose (4.52 mg/m³ air) and in all 10 females on 0.71 mg/m³ air lasting up to 88 days. At the weekends, when no exposure took place, partial recovery with slight non-specific disturbed behaviour was observed.
Growth was reduced from concentrations of 0.09 mg/m³ air upwards in the males, and from 0.71 mg/m³ air in the females (Table B.6.3-8).
No toxicologically relevant changes in the haematological, clinicochemical and urinalysis parameters were found. Determination of the N-demethylase and O-demethylase activities and of cytochrome P 450 yielded no evidence of enzyme induction. The absolute and relative liver weights were reduced in the mid and high dose groups (Table B.6.3-7).
The gross pathological and histopathological examinations did not reveal any evidence of specific organ damage.

Table B.6.3-7: Absolute and relative liver weight

AC (mg/m3) **Sex**	**0 a** **M/F**	**0 v** **M/F**	**0.09** **M/F**	**0.71** **M/F**	**4.52** **M/F**
Liver, absolute Means (mg) + SD (mg) Signi #	 9156/6421 574/505	 8881/6280 783/495	 8336/6157 995/456	 7495/5671 598/512 **/**	 7232/5573 551/517 **/**
Liver, relative Means (mg/100 g bw) + SD (mg/100 g bw) Signi #	 3308/3326 107/235	 3216/3391 201/164	 3232/3329 163/172	 2956/3182 143/155 **/	 3063/3055 154/239 **/*

AC = Analytical concentrations; a = air control ; v = vehicle control; * = p <0.05, ** = p <0.01

Table B.6.3-8: Mean body weight gain

AC (mg/m3) Sex	0 a M/F	0 v M/F	0.09 M/F	0.71 M/F	4.52 M/F
Body weight (g), day 0	192/ 164	192/ 162	191/ 159	186/ 157	190/ 160
Body weight (g), week 12	277/ 193	276/ 185	258*/ 185	253**/ 178**	236**/ 182*

AC = Analytical concentrations; a = air control ; v = vehicle control; * = p <0.05, ** = p <0.01

1. Addendum 15469A (January 22, 1987, TOX9401887): The purpose of this addendum was to provide individual data of histopathological examination.

2. Addendum 15469A (July 30, 1987, TOX9401887): The purpose of this addendum was to provide a more extensive presentation of the methodological details than it was the case in the original report.

Conclusion:

The NOEL of 0.09 mg/m³ air was based on behavioural effects and reductions in growth of male animals exposed to 0.71 mg/m³ air and above.

Re-evaluation by the RMS (2015):

4.52 mg/m³ air: Central nervous effect (agitation, erected tail) in males and female rats. Reduction of final body weight in male and female rats.
0.71 mg/m³ air: Non-specific disturbed behaviour in female rats. Reduction of final body weight in male and female rats.
0.09 mg/m³ air: Reduction of final body weight in male rats.
In the absence of other relevant biological effects in male rats, a NOAEC of 0.09 mg/ m³ air (approx. 0.02 mg/kg bw/d) was determined in this study.
Although a variety of parameters were not examined, the study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph (1996), the study was considered acceptable.

Data point:	KCA 5.3.3
Report:	[redacted]., 1989, TOX9401886 4-Week Inhalation Toxicity Study - Report no.: 18565 (November 28, 1989); [redacted]
Guideline(s):	The test was conducted according to the OECD Guideline for Testing Chemicals no. 412 and complies to Directive 92/69 EEC Method B 8.
Deviations:	The noted deviations do not limit or impair the scientific validity of the study (total bilirubin, albumin, urea, creatinine, alanine aminotransferase, aspartate aminotransferase were not determined, the differential blood count and histopathology was not performed).
GLP:	Yes
Acceptability:	Acceptable

(Dates of exp. work: February - March 1989)

Materials and methods:

Groups of 10 male and 10 female Wistar rats (Bor:WISW; source: [redacted]), were exposed to cyfluthrin (batch no.: 23388016, purity 93.8 %) in aerosol form for 4 weeks (6 h/day, 5 days a week) under dynamic conditions (nose only exposure). The nominal concentrations were 0

(air control)- 0 (vehicle control)- 3 - 30 - 300 mg/m^3 air. The analytical concentrations (AC) were 0.44 - 6.04 - 46.6 mg/m^3 air.
Additional observations: Reflexes: cornea, pinna, myotactic, light, startle and righting, weekly. Rectal temperature: day 3, 10, 13, 17, 20 and 23 (5-20 min after the end of exposure).
Lung function tests: 4 male rats per group were placed in a plethysmograph for 2-3 hours (before and 3 times during the experimental period at weekly intervals) and subsequently nose-only exposed to achieve a total daily exposure of 6 hours. The following lung function parameters were evaluated individually: peak expiratory flow, tidal volume, breaths per minute, respiratory minute volume, inspiratory time and expiratory time. Other measurements: blood gas analysis, haemoglobin concentration, body temperature about 20 to 25 h after the last exposure (5 animals per group per sex). Arterial blood samples were taken from the abdominal aorta under anaerobic conditions. While the animals were anaesthetised, the rectal temperature was measured.
Statistics: Absolute and relative organ weights and blood gas data were analysed using an analysis of variance (ANOVA). Clinical chemistry, haematology and urinalysis data were analysed using the rank test (U-test). Body weights were analysed by both ANOVA and the U-test.

Results and discussions:

Analysis of the test atmosphere: Stable and reproducible conditions of exposure were achieved. The aerosol had a mean mass media aerodynamic diameter (MMAD) of about 1.2 µm. More than 99 % of the aerosol mass may be regarded as readily respirable (particles <5 µm).

Table B.6.3-9: Analytical concentrations (AC) and approximate exposition dose (AED)

AC (mg/m^3 air)	0 a	0 v	0.44	6.04	46.60
AED (mg/kg bw)			0.16	2.2	16.8

(a = air control ; v = vehicle control)

Clinical signs: Mild to moderate clinical signs of a non-specific nature (ruffled coat, hyperactivity, bradypnoea) were observed at the end of exposure to the highest concentration.
Determination of the respiratory parameters revealed, from the dose of 6.04 mg/m³ air onward, a transient reflex bradypnoea (Figure B.6.3-1 and Figure B.6.3-2).
As shown in the next table, a slight, transient reduction in body temperature, as well as a marginal reduction in the leukocyte counts, and (in the males only) a retardation of growth were seen in the same dose range. Slight acidification of the urine was observed at the highest dose, a similar trend also being in evidence at the lower doses.

Table B.6.3-10: General examinations, haematology, clinical chemistry

AC (mg/m^3 air) **Sex**	**0 a** **M/F**	**0 v** **M/F**	**0.44** **M/F**	**6.04** **M/F**	**46.60** **M/F**
Body weight [g] ± SD [g] Signi [#]	230/185 17.5/7.1	233/181 15.9/8.4	227/177 18.8/3.7 /**2	207/172 12/5.8 **/**4	198/175 11.4/8.2 **/**2
Rectal temp.[5)][°C] ± SD [°C] Signi [#]	37.2/37.2 0.39/0.12	37.5/37.9 0.31/0.48 /**1	37.8/38 0.33/0.38 **/**	35.8/35.2 0.52/1.81 **/*	34.5/34.5 1.06/1.67 **/**
Leukocytes [gig/L] ± SD [giga/L] Signi [#]	7.7/6.1 1.66/0.81	6.9/5.4 1.8/1.23	5.9/3.4 0.59/1.24	5.8/2.8 0.59/0.79 /**	4.6/2.3 1.2/0.74 */**

Protein [g/L] ± SD [g/L] Signi #	61.5/62.2 1.97/2.43	61.0/61.4 0.78/2.38	58.8/60.2 2.05/2.34	57.4/58.9 1.90/1.93 **4/	58.2/58.2 1.21/1.88 **4/*2
pH (urine) ± SD Signi #	7.18/7.84 0.63/0.54 ../*1	7.79/6.86 0.79/0.40 ../*1	6.14/6.19 0.32/0.25 **4/**3	6.03/6.35 0.25/0.46 **4/**2	5.72/5.71 0.17/0.14 **/**

(a = air control; v = vehicle control) [# = * p<0.05/** p<0.01]
1) Signi in relation to each other control;
2) Signi only in relation to a;
3) Signi p<0.01 in relation to air control, p< 0.05 in relation to v;
4) Signi p<0.05 in relation to air control, p< 0.01 in relation to v.
5) on day 23

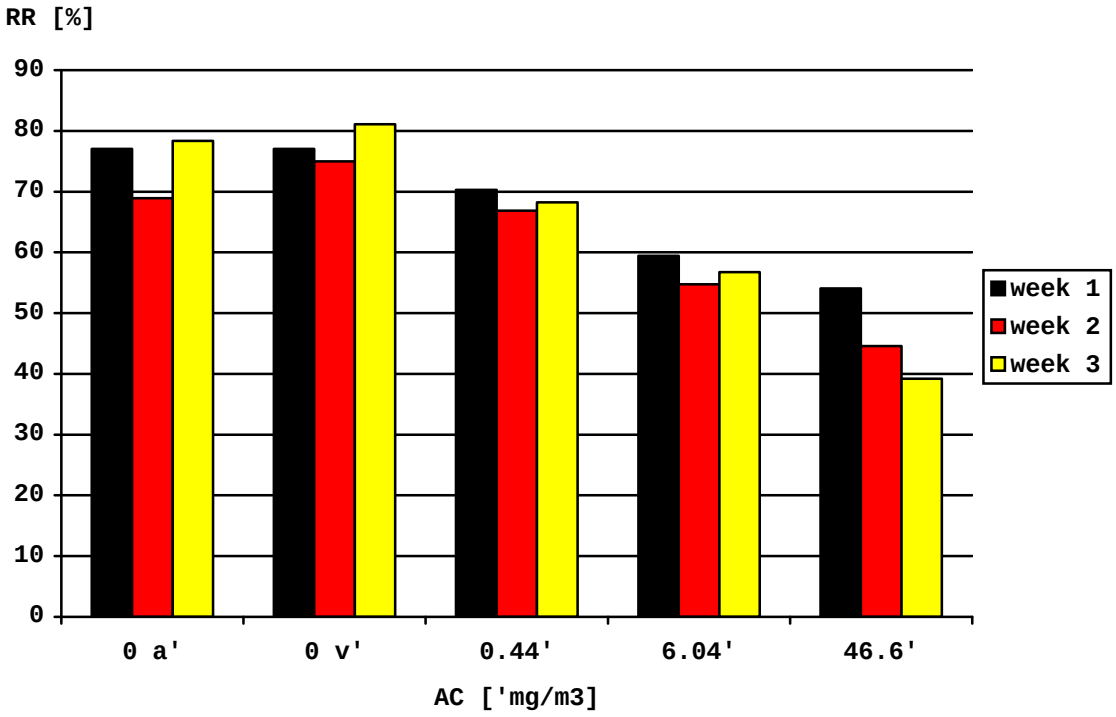


Figure B.6.3-1: Lung function tests - respiratory rate (RR)

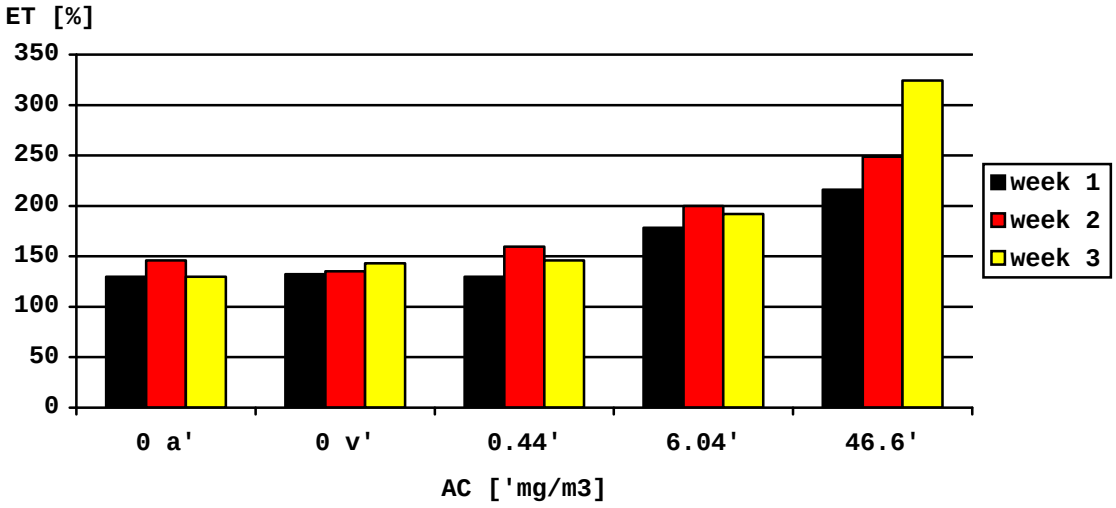


Figure B.6.3-2: Lung function tests - expiratory time (ET)

Reduced thymus weights were observed in both sexes at the highest dose level (Table B.6.3-11).

Table B.6.3-11: Organ weights

AC (mg/m³ air) Sex	0 a M/F	0 v M/F	0.44 M/F	6.04 M/F	46.60 M/F
Thymus, absolute Means [mg] ± SD [mg] Signi #	 270/236 84/49	 242/209 70/61	 234/214 31/52	 150/143 47/19	 124/113 33/36 */*
Thymus, relative Means [mg/100 g bw] ± SD [mg/100 g bw] Signi #	 115/126 30/21	 102/112 23/27	 103/118 7/27	 73/83 22/11	 63/66 14/19

(a = air control ; v = vehicle control) [# = * p<0.05/** p<0.01]

Conclusion:

The NOAEL of 0.44 mg/m³ air was based on transitory bradypnoea, slight transitory reduction of body temperature and (only in male rats) on growth retardation of rats exposed to 6.04 mg/m³ air. This corresponds to a nominal daily dose of about 0.16 mg/kg bw. The marginal changes at the lowest dose level (slight decrease of urine pH and slight increase of body temperature) were not regarded as toxicologically relevant.

Re-evaluation by the RMS (2015):

NOAEL 0.44 mg/m³ air (0.16 mg/kg bw/d).
Although a variety of parameters were not examined, the study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin (1996, ASB2010-10436), the study was considered acceptable.

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599):

Data point: KCA 5.5

Report: [redacted], 1997, TOX9800225:
Technical grade Cyfluthrin (FCR 1272) – A chronic toxicity feeding study in the beagle dog, [redacted] Report-No.: 108007, Study-No.: 94-276-ZR, Bayer File-No.: 8365, unpublished, (Experimental work from .12 October 1994 – 23 October 1995)

Supplemental submission to AC No. 108007 – Technical grade Cyfluthrin (FCR 1272) – A chronic toxicity feeding study in the beagle dog, [redacted], U.S.A., Report-No.: 108007-1, Study-No.: 94-276-ZR, Bayer File-No.: 8365, unpublished

Guideline(s): OECD Guideline No. 452 (adopted 12 May 1981)

Deviations: None that were considered to have compromised the validity of the study results

GLP: Yes

Acceptability: Acceptable

Study completion date: November 10, 1997

Material and Methods:

Test material: Technical grade cyfluthrin, purity: 94.8–95.1 %, batch no.: 4030059/BF9340-71
Test animals: Pure-bred male and female Beagle dogs, age at study initiation not greater than 25 weeks, Source: [redacted]
Technical grade cyfluthrin was administered in the diet to Beagle dogs (4 animals/sex/group) for 12 months at initial nominal concentrations of 0–50–100–360–640 ppm. The selection of doses was based on the results of two former dog studies (6-month study: 0–65–200–600 ppm, NOEL 65 ppm; 12-month study: 0–40–160–600 ppm, NOEL: 160 ppm). The selected intermediate dosage of 360 ppm was expected to confirm a dose response relationship, while 640 ppm was expected to toxicologically stress the animals without influencing survival. However, the high-dose group began to demonstrate severe neurological symptoms in the first few weeks of the study, with one high-dose female requiring sacrifice following a severe seizure episode. Therefore, the high-dose was reduced to 500 ppm beginning on week 8 for the remainder of the study.
In addition to the routine guideline requirements, the study investigated potential cardiac and neurologic effects. Electrocardiography (ECG) and blood pressure (BP) measurements were performed. Neurological examinations, conducted on all animals at approx. 6 months after study start and just prior to study termination, included: peripheral and cranial reflex tests, task performance tests, gait and behavioural observations, as well as rectal temperature measurements. At necropsy, the following organ weights were determined: adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testicles, thymus and thyroid with parathyroid. All tissues and gross lesions from all animals were histopathologically examined.

Results and discussions:

Concentration, stability and homogeneity of the test compound in feed:
The mean treatment concentrations ranged from 98.2–105 % of the nominal concentrations. Based on analytical chemistry determinations, cyfluthrin in the feed was considered to be homogeneously distributed and stable.
Cyfluthrin intake:
The test substance intake is summarised in the table below.

Table B.6.3-12: Dog 12-month study: Calculated test substance intake

Nominal dose levels (ppm)	Average daily consumption of cyfluthrin (mg/kg bw/d)	
	Males	Females
0	0.00	0.00
50	1.36	1.46
100	2.43	3.61
360	10.64	10.74
640/500*	15.47	17.99

This calculation includes the 640 ppm concentration fed during weeks 1-7, since the high-dose was changed on week 8 of the study from 640 ppm to 500 ppm. Therefore, the mean concentration for this level is a time weighted average, calculated to be 523 ppm (105 % of 500 ppm).
Mortality: Two control animals died *in extremis* during the study, a male (ZR0004) on day 318 and a female (ZR0102) on day 210. Necropsy was unremarkable in both animals. The animals had been asymptomatic to trained veterinary technicians prior to the clinical episode. Further investigations indicated that both dogs were genealogically predisposed to seizures and probably died suffering from idiopathic epilepsy. Another high-dose female (ZR4103) suffering from extreme neurological symptoms was sacrificed on day 56 due to animal welfare concerns.
Body weight gain: In evaluation of a possible treatment effect on body weight development, no clear dose-response relationship could be established (see table below). Especially the female dogs had lower body weights at all determination periods from the 50 ppm dose group onwards than the concurrent control females. The loss of the small female control animal that died presumably from idiopathic

epilepsy, caused the remaining three heavier control animals to bias the mean upward, so comparisons to the control group must be qualified accordingly.
A biologically relevant decrease in body weight gain over the 12-mo treatment period within the 640/500 ppm male (-55 %) and female (-54 %) dose groups when compared to concurrent controls that was considered to be compound-related.

Table B.6.3-13: Dog 12-month study: Body weight gains

Time period*	Mean bw gain (g) during the designated study periods at dose (ppm)									
	0	50	100	360	500/640	0	50	100	360	500/640
	Males					Females				
3 mo	3613.7 (100 %)	3013.2 (83 %)	3046.5 (84 %)	3513.0 (97 %)	1629.3 (45 %)	2611.5 (100 %)	1845.7 (71 %)	2053.0 (79 %)	1838.8 (70 %)	920.0 (35 %)
6 mo	4883.0 (100 %)	3520.5 (72 %)	4028.0 (82 %)	4407.7 (9 %)	2350.0 (48 %)	3666.0 (100 %)	1909.5 (52 %)	2939.5 (80 %)	2575.8 (70 %)	1804.7 (49 %)
12 mo	4864.4 (100 %)	3488.2 (72 %)	4404.3 (91 %)	4579.0 (94 %)	2199.8 (45 %)	5220.7 (100 %)	2514.0 (48 %)	3054.3 (59 %)	2775.0 (53 %)	2379.0 (46 %)

* Bw gains were determined for 3 month (Day 0 – Day 91), 6 month (Day 0 – Day 182) and 12 month (Day 0 – Day 364)

Food consumption: There was no compound-related effect on food consumption in any of the dose groups tested.
Clinical symptoms: There were clinical neurology findings in this study related to chronic cyfluthrin administration. The 360 ppm and 640/500 ppm doses groups (both sexes) were affected. In the 360 and 640/500 ppm dose groups, principle findings included gait abnormalities (hypermetria, reluctance to walk) and postural reaction deficits (abnormal head placement during wheelbarrowing and abnormal foot placement during backward stepping, abnormal foot placement during lateral hopping, abnormal hemistanding posture). Gait abnormalities were found at the 6 month and pre-sacrifice examinations. Postural reaction deficits were found at the 6 month and pre-sacrifice examinations.
It appeared that the incidence of gait abnormalities and postural deficits was increased in the 640/500 ppm males and females at both the 6 month and the pre-sacrifice exam intervals when compared to the 360 ppm groups. It appeared that the severity of and extent of abnormalities and deficits was slightly increased in the 640/500 ppm males compared to the 360 ppm males. However, the high-dose females had two individuals (ZR4102 and ZR4103) that had a markedly more severe and extensive neurological syndrome than the 360 ppm females or the other 640/500 ppm females.
There were no changes in rectal body temperature related to chronic cyfluthrin administration, excluding hyperthermia secondary to convulsions. There were no other relevant clinical signs attributed to compound administration.
Electrocardiography (ECG) and blood pressure measurements:
There were no dose-related changes found in the ECG or BP parameters measured in this study.
Clinical chemistry, haematology, urinalyses:
There were no clinical chemistry, plasma cholinesterase, haematology or urinalysis findings that were considered treatment-related or toxicologically relevant.
Ophthalmoscopy:
There were no direct ophthalmological findings related to chronic cyfluthrin administration in this study that were not regarded as variants of normal. However, in one high-dose female, there was a neurological condition that contributed indirectly to opthalmological findings of ptosis, deficits in direct and indirect pupillary responses and protrusion of the nictitating membrane.
Terminal body weight and organ weight changes
There was a non-significant and somewhat inconsistent trend toward decreased terminal body weights in both sexes when compared with controls (see table below). Due to overlaps in individual weights, initial weight spreads, lack of dose relationship and of statistical significance, it can only be suggested that a treatment effect may be present in the 500 ppm group males, which were terminally 18 % lower than controls. Absolute ovary weights from all treated groups were significantly lower than control values, but no significant differences were evident for relative ovary weights.

Table B.6.3-14: Dog 12-month study: Terminal body weight and organ weight changes

	Dose (ppm)				
Parameter	0	50	100	360	640 / 500
MALES					
Terminal bw [g]	14037 (100 %)	13266 (95 %)	13695 (98 %)	14466 (103 %)	11434 (81 %)
FEMALES					
Terminal bw [g]	13503 (100 %)	10383 (77 %)	11098 (82 %)	10495 (78 %)	10296 (76 %)
Ovary abs. wt (g)	1.940 (100 %)	0.889* (46 %)	1.217* (63 %)	1.034* (53 %)	0.789* (41 %)
rel. wt (%)	0.014 ± 0.001 (100 %)	0.009 ± 0.002 (64 %)	0.011 ± 0.003 (79 %)	0.010 ± 0.005 (71 %)	0.008 ± 0.002 (57 %)

Statistics: Anova + Student's t-test (two-sided): * = $p \leq 0.05$

Histopathology: There were no treatment-related microscopic lesions.
The statistically significant decreases in absolute ovary weight changes observed in all treatment groups were likely due to the differences in terminal body weights noted above, as the absolute weights tracked the respective group mean body weights in a near perfect manner. The death of a small control female animal caused the remaining three heavier control animals to bias the mean of the terminal body weight upward and likely caused also a statistical aberration in the absolute ovarian weights. A treatment-related effect on ovary weights was considered to be unlikely in the absence of statistically significant changes in the relative ovary weight, the lack of corresponding histopathological changes, and in the absence of any indication of treatment-related ovary effects from other dog or rodent studies.

Conclusion:

In the 12-month dietary dog study, the NOAEL was established at 100 ppm (equivalent to 2.4 mg/kg bw/d for males and 3.6 mg/kg bw/d for females), based on neurological findings noted at 360 ppm, which demonstrated an intermediate level of toxicity based on findings of gait abnormalities and postural reaction deficits. The MTD (maximum-tolerated-dose) was established at 500 ppm within the limits of animal welfare concerns. The severity of and extent of neurological abnormalities and deficits were increased in the 640/500 ppm dose groups compared to the 360 ppm dose groups.

Re-evaluation by the RMS (2015):

10.64/10.74 mg/kg bw/d (360 ppm): Lower body weights of female dogs, neurological findings: gait abnormalities (hypermetria, reluctance to walk) and postural reaction deficits (abnormal head placement during wheelbarrowing and abnormal foot placement during backward stepping, abnormal foot placement during lateral hopping, abnormal hemistanding posture) in male and female dogs.
15.47/17.99 mg/kg bw/d (640/500 ppm): Lower body weights of male and female dogs, one female dog (ZR4103) suffering from extreme neurological symptoms was sacrificed on day 56 due to animal welfare concerns. Neurological findings: gait abnormalities (hypermetria, reluctance to walk) and postural reaction deficits (abnormal head placement during wheelbarrowing and abnormal foot placement during backward stepping, abnormal foot placement during lateral hopping, abnormal hemistanding posture) in male and female dogs.
The NOAEL is considered to be 100 ppm (2.43/3.61 mg/kg bw/d).
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599), the study was considered acceptable.
In the supplemental submission additional information regarding the cyfluthrin chronic dog study was given: i.e. hereditary idiopathic epilepsy, rationale for high-dose selection of 500 ppm, body weight

parameters in control females, significance of an ovary effect. The assessment of the additional information is included in the original study summary.

Data point:	KCA 5.3.1
Report:	[redacted]., 1996, TOX2001-1769: 21-day dermal toxicity study with technical grade BAYTHROID in rats. [redacted] Study No. 95-122-ES, unpublished (Experimental work from 31 May – 6 July 1995)
Guideline(s):	Yes (OECD Test Guideline No. 410 (adopted May 1981))
Deviations:	None that compromised the validity of the study results
GLP:	Yes
Acceptability:	Acceptable

Material and Methods:

Test material: Cyfluthrin, purity: 95.5–95.9 %, batch no.: 2030025/BF9140-23)
Test animals: Sprague-Dawley rats (Sas: CD(SD)BR), approx. age (Day 0): males 8 weeks, females 10 weeks
Source: [redacted]
Statistical analysis: Body weight, food consumption, terminal organ weights, and clinical pathology: Bartlett's Test; Analysis of Variance (ANOVA); Dunnett's t test Kruskal-Wallis Analysis of Variance pairwise Mann-Whitney U Test.
Ophthalmology data: Chi-Square; one-tailed Fisher's Exact Test
Micropathology data: software from SAS Institute Inc.; DATATOX software. A probability value of $p < 0.05$ was taken as significant for all statistical tests, with the exception of Bartlett's Test in which a value of $p < 0.001$ was accepted.
Groups of 8 male and 8 female Sprague-Dawley rats were treated dermally for 22 and 23 days, respectively with cyfluthrin at doses of 0–100–340–1000 mg/kg bw/d (actual mean doses: 0-113-376-1077 and 1083 mg/kg bw/d in the recovery group), including recovery groups of the control and high dose (two weeks after final application). Doses were administered with a moistened pad (0.4 mL deionized water to the shorn backs such that males received 17 and females received 18 occlusive applications within the 22–23-day treatment, each exposure period lasting at least 6 hours. An additional eight rats of each sex were included with the control and high-dose group and were maintained for two weeks beyond treatment.
The following in-life observations and measurements were taken: mortality (daily), body weight (minimum weekly), food consumption (weekly), clinical observations including irritation at the dose site (daily) and ophthalmologic exams (before study start and shortly before sacrifice). The following terminal post-mortem observations and measurements were performed: haematology, clinical biochemistry, organ weights (ovaries, liver, kidneys, heart, testicles, thyroid with parathyroid, lungs, spleen, adrenals and brain) and incidence of lesions at gross necropsy. Histopathological examination of the following organs was performed in 0 and 1000 mg/kg bw/d rats of both sexes (from both non-recovery and recovery groups): adrenals, brain, heart, kidneys, liver, lungs, ovaries, parathyroid, pituitary, skin (treated), spleen, testicles and thyroid); In addition, "Skin, treated" was examined in 100 and 340 mg/kg bw/d rats of both sexes to establish a no-observed-effect level (NOEL). Gross lesions were processed and examined microscopically in all dose groups.

Results and discussions:

No occurrence of moribundity or incidence of mortality, no ocular abnormalities and no statistically significant decrements in rates of body weight gain were observed.
Food consumption in males and females of the 1000 mg/kg bw/d group was significantly reduced on the first week of treatment (approx. by -13 % and -11 % in males and females, respectively) and per-

sisted during the recovery period.
Compound-related clinical signs like red discharge from the nose (1000 mg/kg bw/d males), scabbing at the dose site (1000 mg/kg bw/d males, ≥340 mg/kg bw/d females) and urine stains (1000 mg/kg bw/d females) were observed.
No variations in clinical chemistry or haematological parameters or in organ weights were considered as a result of treatment. At gross necropsy crusty zones were present on skin from a number of animals at 340, 1000 mg/kg bw/d or 1000 mg/kg bw/d recovery group. Additionally, a discoloured zone was noted on treated skin from one 1000 mg/kg bw/d female and a raised zone in one 340 mg/kg bw/d male was noted. Crusty zones were still present on treated skin in one male and two females of the 1000 mg/kg bw/d group.
Histopathologically epidermal and dermal alterations were in some males and females at 1000 mg/kg bw/d and in one male and one female at 340 mg/kg bw/day and were considered as treatment-related. These microscopic alterations were predominantly characterised by an extensive area of moderate to marked ulceration with bordering epidermis thickened by acanthosis and hyperkeratosis. There was inflammatory cell infiltration in the exposed dermis underlying the ulceration. An accompanying minimal to slight dermal fibrosis in two 1000 mg/kg bw/d females was also noted.
Histopathological alterations from the recovery animals were similar to those observed in non-recovery animals (ulceration, hyperkeratosis, acanthosis, inflammation, and dermal fibrosis). These responses were manifested in one male and two females of the 1000 mg/kg bw/d recovery group. These responses were slightly less severe than from animals sacrificed shortly after treatment indicating some progress towards lesion repair.

Conclusion:

The NOAEL/NOEL for systemic toxicity was established at 340 mg/kg bw/d based on reduced food consumption and red nasal discharge at 1000 mg/kg bw/d. Local adverse skin effects were observed at 340 mg/kg bw/d, so that an overall NOEL for systemic and local toxicity of 100 mg/kg bw/d can be derived.

Re-evaluation by the RMS (2015):

1077 mg/kg bw/d:
Significantly reduced food consumption in males and females on the first week of treatment; dark red discharge from the nose in four males, scabbing at the dose site in males and females, urine stains in females, crusty zones on skin from a number of animals also in the recovery group, discoloured zone in the treated skin in one female, epidermal and dermal alterations in males and females (extensive area of moderate to marked ulceration with bordering epidermis thickened by acanthosis and hyperkeratosis), inflammatory cell infiltration in the exposed dermis underlying the ulceration, dermal fibrosis in two females
376 mg/kg bw/d:
Scabbing at the dose site in females, crusty zones on skin from a number of animals, raised zone in the treated skin in male, epidermal and dermal alterations in one male and one female (extensive area of moderate to marked ulceration with bordering epidermis thickened by acanthosis and hyperkeratosis), inflammatory cell infiltration in the exposed dermis underlying the ulceration
Recovery group 1083 mg/kg bw/d:
Dark red discharge from the nose in two males, histopathological alterations in one male and two females were similar to those observed in non-recovery animals (ulceration, hyperkeratosis, acanthosis, inflammation, and dermal fibrosis), these responses were slightly less severe than from animals sacrificed shortly after treatment indicating some progress towards lesion repair.
The NOAEL for systemic toxicity was established at 376 mg/kg bw/d based on reduced food consumption and red nasal discharge at 1077 mg/kg bw/d. Local adverse skin effects were observed at 376 mg/kg bw/d, so that an overall NOAEL for systemic and local toxicity of 113 mg/kg bw/d can be derived.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599), the study was considered acceptable.

Literature search for the Renewal Assessment Report (RAR):

Data point:	KIIA 5.10
Report:	Bhushan et al. (2013) ASB2015-644 Biochemical and histological changes in rat liver caused by cypermethrin and beta-cyfluthrin. Arh Hig Rada Toksikol 2013;64:57-67
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract: The hepatotoxicity in Wistar rats following sub-acute (1.27, 1.69, 2.53, 5.07 mg/kg bw/d for 7, 14, 21, 28 days) administration of technical-grade cypermethrin and beta-cyfluthrin (95 % purity) was assessed. The acute administration of cypermethrin and beta-cyfluthrin is described in B.6.2 (Acute Toxicity). The assessment was based on hepatic marker enzymes: aminotransferases (AST, ALT), dehydrogenase (LDH), phosphatase (ALP), glycogen, total proteins, total lipids, cholesterol, free fatty acids, and phospholipids. AST, ALT, LDH, total lipids, cholesterol, phospholipids, and free fatty acids in hepatic homogenate increased following pyrethroid administration. In contrast, hepatic proteins, glycogen, and ALP activity decreased due to lysis of structural proteins and leakage of enzymes into the blood stream. Biochemical data were consistent with histological alterations (cytoplasmic vacuolisation, nuclear polymorphism, eccentric nucleus, karyolysis, karyorrhexis, and sinusoidal dilation) in the rat liver. Comparatively greater hepatocellular damage was noted in beta-cyfluthrin than in cypermethrin-treated rats.

Conclusion:

The repeated administration of cypermethrin and beta-cyfluthrin caused histopathological changes in the liver and changes in biochemical liver parameters. The study results are considered to represent supplemental information (e.g. no batch number, no individual values reported).

Data point:	KIIA 5.10
Report:	Bhushan et al. (2010) ASB2015-1098 Beta-Cyfluthrin induced histochemical alterations in the liver of the albino rat. Scand. J. Lab. Anim. Sci. 2010 Vol. 37 No. 2 pp. 61-66
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Not acceptable

Abstract:
Beta-cyfluthrin (batch number and purity not reported) was orally (gavage) administered at doses of 1.68, 2.53, and 5.06 mg/kg bw/d for 7, 14, and 21 days treatment. The acute administration of beta-cyfluthrin is described in B.6.2 (Acute Toxicity). A decrease in hepatic proteins, and an increase of hepatic DNA was reported. Histochemical localisation of hepatic DNA revealed that the centilobular zone to be the most sensitive. Based on these changes and changes of body weight gain (increased in treated animals), liver weight (increased in treated animals) and liver weight/body weight ratio (increased in treated animals) the authors conclude that beta-cyfluthrin has the potential to disrupt normal hepatic functions in mammals.

Conclusion:

The administration of multiple doses of beta-cyfluthrin resulted in an increase in body weight compared to the control group. Consequently, liver weight and liver weight/body weight ratio also increased. The increase in body weight is not understandable and is not in accordance with other repeat-dose studies with beta-cyfluthrin. Clinical observations are not reported, individual values are not given, and the purity of the test compound is not known. It can be assumed that partly the same data as in the paper before are represented. Anyhow, the study is considered not acceptable.

Data point:	KIIA 5.10
Report:	Jebur et al. (2013) ASB2015-921 Selenium Modulates beta-Cyfluthrin-Induced Liver Oxidative Toxicity in Rats. Environ Toxicol 29: 1323–1329, 2014
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
The study was designed to investigate the possibility of beta-cyfluthrin (>99 % pure, vehicle not reported) to induce oxidative stress and biochemical perturbations in rat liver and the role of selenium in alleviating its toxic effects. Male Wister rats were randomly divided into four groups of seven each, group I served as control, group, II treated with selenium (200 mg/kg bw), group III received beta-cyfluthrin (15 mg/kg bw, 1/25 LD_{50}), and group IV treated with beta-cyfluthrin plus selenium (15 and 200 mg/kg bw). Rats were orally administered their respective doses daily for 30 days.
Beta-cyfluthrin induced oxidative stress and elevation in lipid peroxidation leading to perturbations in antioxidant enzymes and liver biomarkers. Selenium administered in combination with beta-cyfluthrin mitigates its hazards. Selenium could be useful to reduce beta-cyfluthrin toxicity by quenching oxidative stress.

Conclusion:

The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Omotuyi et al. (2006) ASB2015-924 Cyfluthrin-induced hepatotoxicity in rats. African Journal of Biotechnology Vol. 5 (20), pp. 1909-1912, 16 October 2006
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Not acceptable

Abstract:
The hepatotoxic effect of continuous administration of cyfluthrin (dissolved in 20 % lecithin in water, purity not reported) was investigated in rats. Rats (*Rattus norvegicus*) were grouped into A (0 ppm) control, B (100 ppm) and C (200 ppm) with the indicated amount of cyfluthrin administered orally (gavage) for 15 weeks.
The hepatotoxicity level was assessed by monitoring the changes in the organ to body weight ratio, micronutrient level (iron, zinc, copper and selenium), the nutritional status (total carbohydrate, total

glucose, total protein, total amino acids, total lipid and total cholesterol), the lipid peroxidation level (glutathione and thiobarbiturate) and the antioxidant enzyme activities (glutathione peroxidase, glutathione reductase, catalase, and glucose-6-phosphate dehydrogenase).

Compared to control values: a decrease in the organ-to-body weight ratio was observed, although not dose-related; zinc, copper and selenium levels increased, although not dose-related; total carbohydrate, glucose, protein, lipid levels decreased, although not dose-related; total amino acid levels increased although not dose-related; no significant difference in cholesterol levels at both dosages; thiobarbiturate level increased and glutathione level decreased dose-related.
Based on these results, the authors concluded that cyfluthrin is potentially hepatotoxic under continuous administration in rats.

Conclusion:

The study design has serious flaws, i.e. body weight gain, which organs are weighed, dosage in mg/kg bw/d, kind of tissue for biochemical investigations are not given. The study results are considered not acceptable.

B.6.4 Genotoxicity

The following new study with the active substance has been conducted on genotoxicity after Annex I inclusion.

- *In vitro* mutagenicity study (Herbold, 2008, ASB2014-7875)

Furthermore, the applicant added a non-GLP study on cyfluthrin to the reference list (Ila et al., 2008, ASB2014-7878). The study reports negative results for bacterial mutagenicity and induction of sister chromatid exchanges, but positive results regarding chromosome aberrations and micronucleus formation. In a position paper Wason (2013, ASB2014-7879) evaluated the results presented as not reliable. The justification for this decision, an expert judgement given by Prof. Kirkland as well as the conclusion drawn by RMS is presented at the end of this chapter.

B.6.4.1 *In vitro* studies

B.6.4.1.1 Gene mutation test (*Salmonella typhimurium*)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.4 /01
Report:	Herbold, B. A. 1986, TOX9550277 FCR 4545 - Salmonella/microsome test for point-mutagenic effect. Report No.: 14187 (January 7, 1986); Bayer AG, Institute of Toxicology, Wuppertal, Germany
Guideline(s):	The test was carried out in compliance with the demands of OECD-Guideline no. 471 (1983).
Deviations:	None. Deviation from recommendation in current guideline (OECD TG 1997) (only listed if relevant for acceptability decision): Less sensitivity of test system as only four instead of five bacterial strains were applied (*E. coli* WP2 or *S. typhimurium* TA102 for specific damages like oxidising mechanisms not included). Efficacy of S9 mix only tested with 2-aminoanthracen (from the today's perspective a further mutagen is recommended).
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered supplementary.

(Dates of exp. work: 1985, July).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity 98.5 %) was tested in concentrations up to 12500 µg/plate (first experiment) or 8000 µg per plate (second experiment) by means of the direct plate incorporation method. The stability was tested before the mutagenicity experiment. Bacterial cultures of *Salmonella typhimurium* LT2 mutants TA98, TA100, TA1535 and TA 1537 were grown up by established procedures. The original strains were obtained from Prof. Bruce Ames (arrival at the Institute of Toxicology on 8th November 1982). The test material as well as the positive control substances were dissolved in dimethylsulphoxide (DMSO) which was also used as negative control compound. Direct acting posi-

tive control compounds were sodium azide (for TA1535, 10 µg/plate), nitrofurantoin (TA100, 0.2 µg/plate), 4-nitro-1,2-phenylene diamine (TA1537, 10 µg/plate and TA98, 0.5 µg/plate). 2-aminoanthracene (all four strains, 3 µg/plate) served as positive control substance requiring metabolic activation. The bacteria suspensions used for the study derived from 17-hour cultures (37 °C, 90 rpm) in nutrient broth. In both trials, four plates per strain and dose were tested with and without metabolic activation. For the activation experiment, S-9 mix was derived from adult male Sprague Dawley rats. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254 (500 mg/kg bw dissolved in peanut oil). The liver supernatant fluid was prepared and combined with an appropriate cofactor solution according to established procedures. After an incubation period of 48 h at 37 °C, the colonies were counted.
Evaluation criteria: A reproducible dose-related increase in mutant counts for at least one strain is considered positive. In addition, about double the negative control count should be reached.

Results and discussions:

Both trials did not reveal evidence of bacteriostatic activity in any of the four tester strains up to 12500 µg/plate.
In the first experiment using concentrations of 20, 100, 500, 2500, and 12500 µg/plate, substance precipitation was observed at the top dose level. Thus, mutant count was not assessable at this dose level. Up to the next lower dose of 2500 µg per plate, no significant increase in mutant number was noted.
In the second trial (500-1000-2000-4000-8000 µg/plate), slight precipitation was apparent at the highest dose. However, counting of mutant colonies was still possible. There was no increase in mutant number at any of the dose levels neither in the presence nor in the absence of a metabolically activating system (Table B.6.4-1, to Table B.6.4-4). In contrast, positive control compounds gave the expected increase confirming the sensitivity of the test system.

Table B.6.4-1: First trial - Without Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]			
		Base-pair substitution type		Frameshift type	
		TA1535	TA100	TA1537	TA98
–	0	16 ± 7	71 ± 16	10 ± 4	14 ± 2
–	20	23 ± 5	79 ± 14	7 ± 1	22 ± 3
–	100	18 ± 6	79 ± 10	9 ± 3	16 ± 5
–	500	21 ± 4	75 ± 5	12 ± 4	16 ± 5
–	2500	20 ± 7	89 ± 8	7 ± 3	14 ± 4
–	12500	P*	P	P	P
Pos. controls -S9	Name	Sodium azide	Nitrofurantoin	4-Nitro-1,2-phenylene diamine	
	Conc. [µg/plate]	10	0.2	10	0.5
	Revertants per plate	1024 ± 145	333 ± 34	32 ± 6	57 ± 11

*P: precipitation

Table B.6.4-2: First trial - With Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]			
		Base-pair substitution type		Frameshift type	
		TA1535	TA100	TA1537	TA98
+	0	42 ± 7	149 ± 12	10 ± 4	42 ± 11
+	20	38 ± 7	122 ± 4	11 ± 44	42 ± 7

+	100	35 ± 11	120 ± 21	12 ± 3	52 ± 8
+	500	33 ± 8	123 ± 14	10 ± 3	44 ± 8
+	2500	37 ± 1	121 ± 18	8 ± 3	34 ± 7
+	12500	P*	P	P	P
Pos. controls -S9	Name	2-Aminoanthracen			
	Conc. [µg/plate]	3			
	Revertants per plate	331 ± 34	1631 ± 80	71 ± 6	390 ± 28

*P: precipitation

Table B.6.4-3: Second trial - Without Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]			
		Base-pair substitution type		Frameshift type	
		TA1535	TA100	TA1537	TA98
–	0	20 ± 3	88 ± 17	6 ± 1	21 ± 4
–	500	18 ± 3	79 ± 17	6 ± 2	17 ± 7
–	1000	20 ± 4	67 ± 12	7 ± 4	16 ± 5
–	2000	21 ± 4	72 ± 13	11 ± 1	19 ± 3
–	4000	19 ± 5	70 ± 10	9 ± 2	13 ± 4
–	8000	16 ± 3 P*	71 ± 14 P	8 ± 4 P	15 ± 2
Pos. controls -S9	Name	Sodium azide	Nitrofurantoin	4-Nitro-1,2-phenylene diamine	
	Conc. [µg/plate]	10	0.2	10	0.5
	Revertants per plate	517 ± 7	244 ± 5	57 ± 9	52 ± 14

*P: precipitation

Table B.6.4-4: Second trial - With Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]			
		Base-pair substitution type		Frameshift type	
		TA1535	TA100	TA1537	TA98
+	0	13 ± 1	117 ± 8	13 ± 4	21 ± 4
+	500	11 ± 2	109 ± 12	9 ± 3	22 ± 3
+	1000	13 ± 5	109 ± 24	7 ± 3	25 ± 5
+	2000	11 ± 4	100 ± 16	9 ± 2	24 ± 2
+	4000	12 ± 4	85 ± 7	8 ± 2	21 ± 6
+	8000	10 ± 3 P*	99 ± 21 P	5 ± 2 P	12 ± 3 P
Pos. controls -S9	Name	2-Aminoanthracen			
	Conc. [µg/plate]	3			
	Revertants per plate	223 ± 46	2629 ± 186	454 ± 51	1116 ± 57

*P: precipitation

Conclusion:

The Salmonella microsome test did not provide any indication of beta-cyfluthrin exhibiting a mutagenic effect on the tester strains used in assessable doses of up to and including 8000 µg/plate.

Re-evaluation by the RMS (2015):

The study is now considered to be supplementary. In the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered to be acceptable. The reasons for the supplementary status are deviations from the current guideline listed below. It should be noted that the materials and methods section as well as the results section were revised.
The study presented is generally in agreement with OECD-Guideline no. 471 (adopted in May 26, 1983). As the guideline already existed when the study was performed, it remains unclear, why the study is not referred to this guideline.
Under the conditions of the study and based on the information given in the report, no mutagenic effect of the test compound was observed in all tested bacterial strains with/without S9 mix up to the highest achievable concentration (no or only slight precipitation). Neither dose-dependent duplication of revertants/plate nor biologically relevant increase in revertants/plate (in comparison to the negative control) occurred. However, positive controls showed strong increases in the number of revertants/plate.
From the today's perspective, the test performance would not be sufficient to draw a final conclusion concerning mutagenicity. The current OECD-Guideline (July 21st, 1997) requires the application of five instead of four bacterial strains. Due to the finding that the standard *S. typhimurium* strains TA1535, TA1537, TA98, TA100 as well as TA97 and TA97a may not detect all mutagens (e.g. oxidising or cross-linking substances), *E. coli* WP2 or *S. typhimurium* TA102 should be additionally included in the test protocol. Furthermore, the efficacy of S9 mix should not be solely tested with 2-aminoanthracen, but also with another mutagen being prone to metabolic activation by microsomal enzymes (e.g. benzo[*a*]pyrene). The guideline also demands the listing of historical negative and positive controls in the test report for reproducibility confirmation.

The applicant disagreed with the acceptability status (supplemental). To their opinion the two deviations listed by the RMS are not critical enough to have an impact on the outcome of the study.
However, due to the deviations listed above, the study is considered supplementary by the RMS. It is noted that this decision has no impact on the overall conclusion that beta-cyfluthrin has no genotoxic potential.

Studies submitted with the dossier for the Renewal Assessment Report:

Data point:	KCA 5.4.1 /04
Report:	Herbold, B. 2008, ASB2014-7875 Beta-cyfluthrin - [Project: FCR 4545 (AE 1430672)] – Salmonella/Microsome Test Plate Incorporation and Preincubation Method - TXFRL002. Report No: AT04856 (September 22, 2008), M-308394-01-1; Bayer HealthCare AG, PH-R&D Toxicology, Wuppertal, Germany
Guideline(s):	The test was carried out in compliance with the demands of OECD-Guideline no. 471 (1997).
Deviations:	Efficacy of S9 mix only tested with 2-aminoanthracen (from the today's perspective a further mutagen is necessary) (recommended in OECD TG 1997). Assignments for bacteriotoxic and mutagenic effects in the tables confusing (or even false).

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is considered supplementary.

(dates of exp. work: July 2008 – August 2008).

Materials and methods:

Beta-cyfluthrin (Lot/Batch no.: RD/Reg05-B-34-162/S-1, purity 98.6 %) was initially investigated using the Salmonella/microsome plate incorporation test for point mutagenic effects in doses of up to and including 5000 µg/plate on five *Salmonella typhimurium* strains (TA1535, TA100, TA1537, TA98, and TA102). Original strains were obtained from Prof. Bruce Ames and arrived at Toxicology, Bayer HealthCare AG, on August 15, 1997. The bacteria suspensions used for the study derived from 17-hour cultures (37 °C, 90 rpm) in nutrient broth. The independent repeat was performed as pre-incubation with doses of up to and including 3200 µg/tube.
The stability of the test item in the vehicle (0.02–250 mg/mL) was analytically verified for up to 45 h.
The S9 mix was made from the livers of at least six adult male Sprague Dawley rats. For enzyme induction, the animals received a single intraperitoneal injection of Aroclor 1254, dissolved in corn oil, at a dose of 500 mg/kg bw, five days prior to sacrifice.
The amount of solvent for the test substance and for the controls was 0.1 mL/plate.
The results of the first experiment were considered as a pre-test for toxicity. Doses of repeats were chosen on the basis of the results obtained in the first experiment.
The independent repeat was performed as pre-incubation 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; three plates were used for each strain and dose. An equal number of plates, filled with the solvent minus the test substance, comprised the negative control. Each positive control also contained three plates per strain. The tests were performed both with and without S9 mix. The count was made after the plates had been incubated for 48 h at 37 °C.
The following doses of beta-cyfluthrin were evaluated in the first test: 16, 50, 158, 500, 1581, 5000 µg/plate (plate incorporation). Due to the substance's toxicity, doses up to 3200 µg per tube (100, 200, 400, 800, 1800, 1600, 3200 µg/tube) were chosen for the repeat tests (pre-incubation).
The following doses were used for the positive controls:

Table B.6.4-5: Dosing schedule

Positive control	Concentrations [µg/plate]	Vehicle	Remarks
Sodium azide	10, 20	DMSO	TA1535, -S9
Nitrofurantoin	0.2, 0.4	DMSO	TA100, -S9
4-Nitro-1,2-phenylene diamine	10, 20	DMSO	TA1537, -S9
4-Nitro-1,2-phenylene diamine	0.5, 1	DMSO	TA98, -S9
Mitomycin C	0.2, 0.4	Water	TA102, plate incorporation, -S9
Cumene hydroperoxide	50, 75	DMSO	TA102, pre-incubation, -S9
2-Aminoanthracene	3, 6	DMSO	All strains, +S9

Acceptance criteria:
The following criteria determined the acceptance of an assay:
The negative controls had to be within the expected range, as defined by published data (e.g. Maron and Ames, 1983) and/ or the laboratory's own historical data.
The positive controls had to show sufficient effects, as defined by the laboratory's experience.
Titer determinations had to demonstrate sufficient bacterial density in the suspension.
Assessment Criteria:

A reproducible and dose-related increase in mutant counts of at least one strain is considered to be a positive result. For TA1535, TA100 and TA98 this increase should be about twice that of negative controls, whereas for TA1537, at least a threefold increase should be reached. For TA102 an increase of about 100 mutants should be reached. Otherwise, the result is evaluated as negative.

Results and discussions:

Substance precipitation occurred at the dose 1581 µg/plate and above.
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 was applied both to the tests with and without S9 mix (Table B.6.4-6 and Table B.6.4-8) up to the highest achievable concentration (without precipitation, without bacteriotoxic effect) and was confirmed by the results of the pre-incubation trials (Table B.6.4-7 and Table B.6.4-9).
The positive controls sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide 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, beta-cyfluthrin was considered to be non-mutagenic without and with S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

Table B.6.4-6: Plate incorporation method - Without Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]									
		Base-pair substitution type						Frameshift type			
		TA1535		TA100		TA102		TA98		TA1537	
–	0	13 ± 3		111 ± 14		183 ± 9		16 ± 5		6 ± 1	
–	16	10 ± 6		106 ± 9		169 ± 5		16 ± 5		6 ± 2	
–	50	11 ± 5		129 ± 12		151 ± 18		17 ± 3		5 ± 1	
–	158	7 ± 2		117 ± 12		204 ± 10		23 ± 14		6 ± 2	
–	500	13 ± 7		88 ± 8		150 ± 8		13 ± 6		6 ± 2	
–	1581	12 ± 2	P*	115 ± 9	P	126 ± 7	P	14 ± 1	P	4 ± 1	P
–	5000	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P
Pos. controls -S9	Name	Sodium azide		Nitrofurantoin		Mitomycin C		4-Nitro-1,2-phenylene diamine			
	Conc. [µg/plate]	10	20	0.2	0.4	0.2	0.4	0.5	1	10	20
	Revertants per plate	790 ± 94	992 ± 48	438 ± 55	687 ± 38	951 ± 86	1147 ± 89	133 ± 15	284 ± 22	74 ± 8	161 ± 11

*P: precipitation

Table B.6.4-7: Pre-incubation method – Without Metabolic activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]									
		Base-pair substitution type						Frameshift type			
		TA1535		TA100		TA102		TA98		TA1537	
–	0	8 ± 3		140 ± 2		213 ± 20		14 ± 4		7 ± 1	
–	100	10 ± 1		141 ± 10		201 ± 28		14 ± 6		7 ± 2	
–	200	12 ± 2		128 ± 9		161 ± 11		17 ± 1		8 ± 3	

–	400	10 ± 2		121 ± 17		175 ± 15		13 ± 4		9 ± 4	
–	800	7 ± 3		55 ± 3		148 ± 15		14 ± 2		7 ± 3	
–	1600	5 ± 2	P*	47 ± 7	P	125 ± 17	P	11 ± 3	P	5 ± 2	P
–	3200	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P
Pos controls -S9	Name	Sodium azide		Nitrofurantoin		Cumene hydroperoxide		4-Nitro-1,2-phenylene diamine			
	Conc. [µg/tube]	10	20	0.2	0.4	50	75	0.5	1	10	20
	Revertants per plate	775 ± 30	897 ± 65	557 ± 23	983 ± 30	513 ± 46	599 ± 42	166 ± 18	335 ± 36	129 ± 17	264 ± 7

*P: precipitation

Table B.6.4-8: Plate incorporation method – With Metabolic activation

S9 Mix	**Test substance concentration [µg/plate]**	**Number of revertants [mean number of colonies per plate ± SD]**									
		Base-pair substitution type						Frameshift type			
		TA1535		TA100		TA102		TA98		TA1537	
+	0	9 ± 3		158 ± 13		221 ± 58		36 ± 9		10 ± 1	
+	16	7 ± 2		139 ± 36		188 ± 33		27 ± 4		7 ± 1	
+	50	8 ± 2		170 ± 7		178 ± 17		29 ± 4		5 ± 3	
+	158	8 ± 2		155 ± 20		204 ± 17		31 ± 9		4 ± 2	
+	500	7 ± 2		152 ± 3		147 ± 47		23 ± 4		7 ± 2	
+	1581	8 ± 1	P*	110 ± 18	P	113 ± 23	P	11 ± 4	P	6 ± 1	P
+	5000	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P
Pos controls +S9	Name	2-Aminoanthracene									
	Conc. [µg/plate]	3	6	3	6	3	6	3	6	3	6
	Revertants per plate	103 ± 11	65 ± 0	1767 ± 366	1593 ± 215	768 ± 129	2100 ± 321	1352 ± 134	1628 ± 196	156 ± 12	59 ± 25

*P: precipitation

Table B.6.4-9: Pre-incubation method - With Metabolic Activation

S9 Mix	Test substance concentration [µg/plate]	Number of revertants [mean number of colonies per plate ± SD]									
		Base-pair substitution type						Frameshift type			
		TA1535		TA100		TA102		TA98		TA1537	
+	0	8 ± 1		177 ± 18		269 ± 33		27 ± 4		9 ± 2	
+	100	8 ± 2		187 ± 15		284 ± 32		23 ± 5		9 ± 1	
+	200	9 ± 5		196 ± 16		301 ± 28		24 ± 4		7 ± 1	
+	400	9 ± 2		195 ± 28		255 ± 18		24 ± 3		6 ± 2	
+	800	7 ± 3		119 ± 17		156 ± 12		20 ± 2		8 ± 1	
+	1600	4 ± 2	P*	54 ± 10	P	122 ± 7	P	16 ± 4	P	6 ± 2	P
+	3200	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P	0 ± 0	P
Pos controls +S9	Name	2-Aminoanthracene									
	Conc. [µg/plate]	3	6	3	6	3	6	3	6	3	6
	Revertants per plate	84 ± 16	47 ± 17	2544 ± 124	2172 ± 145	899 ± 90	2078 ± 175	951 ± 17	1515 ± 169	306 ± 24	57 ± 37

*P: precipitation

Conclusion:

The presented mutagenicity tests based on OECD-Guideline no. 471 (adopted July 21st, 1997) are considered supplementary.
Under the conditions of the study and based on the information given in the report, the test compound did not show mutagenic potency in all five strains tested. After performing the plate incorporation and preincubation test with/without S9 mix no dose-dependent or biologically relevant increases in the number of revertants/plate in comparison to the negative controls were observed up to the highest achievable concentration.
However, strong increase in the number of revertants/plate was observed in the positive controls. Apart from that, the presentation of mean values instead of medians for the historical data would facilitate the comparison of the data. Furthermore, the efficacy of S9 mix was only tested with 2-aminoanthracen even though a second mutagen being prone to metabolic activation by microsomal enzymes (e.g. benzo[*a*]pyrene) should be also tested according to the current test guideline.
At the end of the report tables with the raw data are presented. The data are assessed inconsistently to the described results regarding mutagenicity or bacteriotoxicity (maybe writing errors).
As the limiting factor for the dose range in the mutagenicity tests is the precipitation of the test substance (which seems to take place at similar dose level where toxicity seems to be apparent), the outcome of the study is rather not affected.

The applicant disagreed with the acceptability status (supplemental). To their opinion the two deviations listed by the RMS are not critical enough to have an impact on the outcome of the study. However, due to the deviations listed above, the study is considered supplementary by the RMS. It is noted that this decision has no impact on the overall conclusion that beta-cyfluthrin has no genotoxic potential.

B.6.4.1.2 Gene mutation test (HGPRT assay on chinese hamster ovary cells)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.4.1 /02
Report:	Lehn, H. 1988, TOX9550280 Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay *in vitro*. Report no. 16835 (June 27, 1988), Study no. T 8025687; Bayer AG, Institute for Toxicology, Wuppertal, Germany
Guideline(s):	The study met the criteria of OECD-Guideline No.476 (1984).
Deviations:	More than one dose above precipitation observation applied (deviation from recommendation in OECD TG 1984). Single data (*e.g.* colony number for the calculation of the "survival to treatment") are missing (deviation from recommendation in OECD TG 1984). Deviation from current guideline (OECD TG 1997) (only listed if relevant for acceptability decision): None.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(Dates of exp. work: June - October 1987).

Materials and methods:

The frequency of mutations at the hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT) locus of chinese hamster ovary (CHO) cells exposed to beta-cyfluthrin (batch no.: 16001/85, purity: 99.6 %) in the presence and absence of metabolic activation by S-9 mix (supplied by Litton Bionetics, Ltd., source: male Sprague Dawley rats induced by Aroclor 1254) was examined. The stability of the test compound in the vehicle (DMSO) was tested before performing the experiment (0.5–50 mg/mL). To prevent impact of DMSO on cell growth or viability, the final concentration of the vehicle in the medium was ≤1 % (v/v). The CHO-K1-BH_4 subclone was used in the study, kind gift from Dr. A. W. Hsie, Oak Ridge National Laboratory, Oak Ridge, Tennessee. The cultures were periodically tested for karyotype stability and absence of contamination with mycoplasma. During the whole experiment cells were maintained in hypoxanthin-free Man's F12 medium (Biochrom KG or Gibco). It was supplemented with *L*-glutamine, penicillin, streptomycin and heat-inactivated foetal calf serum.
CHO cells (monolayer) were exposed to the test compound for 5 h at dosages from 20 up to 100 µg/mL medium. These dose levels were established on the basis of a preliminary cytotoxicity test. Untreated cells served as negative control. A solvent control was also included in this study. Ethylmethanesulphonate (EMS, 1200 µg/mL) as direct acting mutagen and dimethylbenzanthracene (DMBA, 20 µg/mL, applied in the presence of S-9 mix) served as positive control substances. Testing was carried out in two separate experiments. In both trials, two cultures were used per dose level.
Thereafter, cells were washed with PBS, trypsinised and replated in culture medium for determination of (1) survival to treatment and (2) cloning efficiency as well as for (3) counting the 6-thioguanine resistant colonies after exposure to 6-thioguanine.
Evaluation criteria: An assay is considered positive if a dose dependent and reproducible increase in mutant frequency was observed for at least three doses or when such a reproducible increase was counted in a single dose near the highest concentration tested. A mutagenic response should be at least twice that of the negative controls. The test result would be considered suspicious if a significant increase in mutant frequency was noted in one or more doses but lacking any dose relation.

Results and discussions:

In a preliminary test, no evidence of cytotoxicity was noted up to the highest tested concentrations of 40 µg/mL (with S-9 mix) or 100 µg/mL (without S-9 mix). However, precipitation of the test article was occurring at concentrations above 40 µg/mL. Nevertheless, the test was conducted with and without S-9 mix at the same dose levels ranging from 20 to100 µg/mL.

No significant cytotoxic effects were observed after treatment with the test substance as there was no decrease in relative survival and population growth (Table B.6.4-10, to

Table B.6.4-13).
There was no dose related increase in the number of mutants in the beta-cyfluthrin treated cell cultures neither in the presence nor in the absence of S-9 mix.
Isolated findings of a statistically significant increase in mutation frequency over the concurrent vehicle control were noted at 40 and 50 µg/mL in the first non-activation trial and at 20 µg/mL following activation. However, these results were noted in one culture only. They were not confirmed in the second experiment. Therefore, these findings were considered spurious. The positive control substances gave the expected marked increase in mutation rate.

Table B.6.4-10: Results trial 1 – Without S9 mix

Substance tested	Concentration [µg/ml]	Survival to treatment		Relative population growth [% of control][b]	Absolute cloning efficiency ± SD [%][c]	Mutant frequency [* 10^{-6}][d]
		Mean colony number ± SD	Percent vehicle control[a]			
Negative control[e]	-	[g]	-	[g]	-	-
	-			139.8	67.5 ± 5	1.9
Vehicle control[f]	-	190 ± 10	100	100.0	69.0 ± 3	3.6
	-			100.0	76.5 ± 1	2.5
EMS	1200	[g]	-	11.4	12.5 ± 2	390.0**
	1200			20.6	13.0 ± 0	500.0**
Test substance	20	151 ± 5	79.5	63.3	58.5 ± 6	3.2
	20			159.9	68.0 ± 5	4.6
	25	155 ± 21	81.6	143.9	59.7 ± 10	2.1
	25			226.2	54.0 ± 6	5.8
	40	179 ± 22	94.2	109.4	58.0 ± 1	6.5
	40			102.0	61.5 ± 13	24.4**
	50	171 ± 14[h]	90.0	66.6	62.5 ± 8	5.0
	50			65.2	63.0 ± 27	64.5**
	80	154 ± 21[h]	81.1	91.4	55.5 ± 9	5.6
	80			66.5	62.5 ± 5	4.0
	90	[g, h]	-	178.1	51.0 ± 7	2.5
	90			251.9	61.0 ± 5	4.1
	100	142 ± 91[h]	74.6	58.2	51.0 ± 7	2.5
	100			60.1	61.0 ± 5	4.1

[a] Relative survival [%]
= (average no. of colonies per treated culture/average no. of colonies per vehicle control dish) * 100; 200 cells/dish seeded

[b] Relative population growth [%]
= (treated culture population increase over the expression period/vehicle control population increase over the

expression period)*100
[c] Absolute cloning efficiency [%]; 200 cells/dish seeded
= (average no. of viable colonies per dish/200) *100; 200 cells/dish were seeded for cloning efficiency
[d] mutant frequency [10^6 clonable cells]
= total mutant clones/(no. of dishes * 0.002 * absolute cloning efficiency); 2*10^5 cells/dish were seeded for mutant selection, 8 dishes were used for each concentration
[e] negative control: culture medium
[f] vehicle control: culture medium with 1 % (or less) vehicle
[g] no evaluatuion possible as at least one dish was lost due to contamination
[h] precipitation of the test substance
** significant increase, $p < 0.01$
* significant increase, $p < 0.05$

Table B.6.4-11: Results trial 2 – Without S9 mix

Substance tested	Concentration [µg/ml]	Survival to treatment		Relative population growth [% of control][b]	Absolute cloning efficiency ± SD [%][c]	Mutant frequency [* 10^{-6}][d]
		Mean colony number ± SD	Percent vehicle control[a]			
Negative control[e]	-	[g]	-	102.4	73.0 ± 4	0.9
	-			95.7	82.8 ± 7	4.5
Vehicle control[f]	-	[g]	-	100.0	75.5 ± 4	5.8
	-			100.0	69.0 ± 4	4.5
EMS	1200	[g]	-	20.9	24.3 ± 4	622.1**
	1200			17.1	34.7 ± 8	443.1**
Test substance	20	[g]	-	97.3	69.8 ± 7	8.1
	20			82.7	81.5 ± 8	0.8
	25	[g]	-	69.0	82.3 ± 2	0.8
	25			60.6	90.5 ± -	0.7
	30	[g]	-	89.0	76.3 ± 5	4.9
	30			71.1	65.8 ± 1	5.7
	35	[g]	-	105.1	74.3 ± 4	5.9
	35			110.7	65.0 ± 3	10.0
	40	[g]	-	90.5	83.2 ± 3	4.5
	40			85.4	62.3 ± 8	4.0
	50	[g, h]	-	71.3	92.3 ± 5	0.7
	50			97.9	68.8 ± 4	4.5
	100	[g, h]	-	65.5	93.2 ± 7	1.3
	100			58.2	101.0 ± 6	3.1

For explanation of footnotes see Table B.6.4-10.

Table B.6.4-12: Results trial 1 – With S9 mix

Substance tested	Concentration [µg/ml]	Survival to treatment		Relative population growth [% of control][b]	Absolute cloning efficiency ± SD [%][c]	Mutant frequency [* 10^{-6}][d]
		Mean colony number ± SD	Percent vehicle control[a]			
Negative control[e]	-	129 ± 3	73.7	113.0	81.7 ± 8	4.6
	-			100.0	80.0 ± 6	3.1

Vehicle control[f]	-	175 ± 23	100.0	100.0	90.2 ± 7	6.2
	-			100.0	83.0 ± 8	6.0
DMBA	20	168 ± 27	96.0	63.2	69.0 ± 4	41.7**
	20			86.3	67.5 ± 3	52.8**
Test substance	20	188 ± 23	107.1	88.1	82.8 ± 11	9.1
	20			109.2	84.3 ± 13	5.2
	25	143 ± 6	81.7	103.3	86.2 ± 13	5.8
	25			99.9	76.8 ± 7	9.0
	30	140 ± 2	80.1	70.0	79.8 ± 5	4.7
	30			89.4	76.8 ± 6	4.9
	35	146 ± 7	83.2	94.9	92.3 ± 3	8.8
	35			85.2	78.5 ± 1	12.7
	40	124 ± 4	70.5	91.8	87.3 ± 8	7.9
	40			100.1	85.0 ± 4	14.0
	50	140 ± 8[h]	80.0	90.9	93.8 ± 6	1.3
	50			88.1	76.7 ± 10	8.1
	100	153 ± 6[h]	87.7	124.4	79.0 ± 12	8.7
	100			110.6	70.2 ± 9	8.0

For explanation of footnotes see Table B.6.4-10.

Table B.6.4-13: Results trial 2 – With S9 mix

Substance tested	**Concentration [µg/ml]**	**Survival to treatment**		**Relative population growth [% of control][b]**	**Absolute cloning efficiency ± SD [%][c]**	**Mutant frequency [* 10^{-6}][d]**
		Mean colony number ± SD	**Percent vehicle control[a]**			
Negative control[e]	-	194 ± 15	101.0	103.6	63.0 ± 4	30.6
	-			67.6	76.2 ± 6	35.6
Vehicle control[f]	-	192 ± 13	100.0	100.0	78.3 ± 5	30.9
	-			100.0	69.8 ± 6	28.7
DMBA	20	92 ± 12	48.1	60.0	75.8 ± 3	163.3**
	20			39.5	64.2 ± 13	211.3**

Test substance	20	157 ± 19	81.6	79.0	74.5 ± 1	38.9
	20			62.1	64.3 ± 10	62.2**
	25	175 ± 7	91.3	91.2	62.0 ± 7	36.3
	25			57.3	79.8 ± 6	36.8
	30	177 ± 7	92.4	88.1	86.5 ± 3	31.2
	30			77.7	67.8 ± 4	34.8
	35	191 ± 13	99.7	111.6	66.8 ± 1	38.7
	35			64.3	77.8 ± 7	38.6
	40	167 ± 7	87.0	82.3	66.8 ± 5	32.9
	40			69.1	64.2 ± 7	32.1
	50	179 ± 20[h]	93.1	113.1	61.7 ± 9	40.5
	50			73.1	70.5 ± 2	37.5
	100	146 ± 9[h]	75.9	93.7	62.7 ± 3	36.5
	100			81.3	66.2 ± 12	41.6

For explanation of footnotes see Table B.6.4-10.

Conclusion:

Beta-cyfluthrin was found negative in the HGPRT assay in CHO cells under the test conditions chosen.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results section were revised.
The study is in compliance with the OECD-Guideline no. 476 (adopted April 4th, 1984). It remains unclear why this guideline (which already existed at the date of experimental work) was not mentioned in the study report.
Under the conditions of the study and based on the information given in the report, the test compound generally did not induce a statistically significant increase in the frequency of mutant colonies (with/without S9 mix) in comparison to the negative or vehicle control. Statistically significant increases in mutation frequency were only observed in three single cases. However, these findings were not confirmed in the second experiment and underlie no dose-response relationship. The data of the positive controls were in agreement with the historical data presented in the report.
The highest dose for the HPRT test was chosen after performing a preliminary test. No cytotoxicity was observed up to the highest concentration but the test substance precipitated at concentrations above 40 µg/mL. In contrast to this, the study report does not include a rationale for extending the dose range up to a top dose of 100 µg/mL. Single data (e.g. colony number for the calculation of the "survival to treatment") are missing in the report.

B.6.4.1.3 Chromosomal aberration assay (cytogenetic study on human lymphocytes)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.4.1 /01
Report:	Herbold, B. A. 1988, TOX9550281 FCR 4545 – *In vitro* cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. Report No.: 17116 (June 6, 1988), Study No. T 5027277; Bayer AG, Institute for Toxicology, Wuppertal, Germany
Guideline(s):	The test procedure complied to a great extent to OECD-Guideline No. 473 (1983).
Deviations:	Dose selection for the study not in agreement with cytotoxicity and precipitation (deviation from recommendation in OECD TG 1983). Only one sampling and preparation time (deviation from recommendation in OECD TG 1983). Test substance not diluted in growth medium (deviation from recommendation in OECD TG 1983). Lack of documentation (*e.g.* CO_2 content) (deviation from recommendation in OECD TG 1983). Deviation from recommendation in current guideline (OECD TG 2014) (only listed if relevant for acceptability decision): Only 200 instead of at least 300 metaphases per concentration and control scored. Exposure time with S9 mix is too short.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is not acceptable.

(Dates of exp. work: February - April 1988).

Materials and methods:

Human lymphocytes were obtained from the blood of two healthy donors (one male and one female). The culture preparation was according to the method of Moorhhead et al. (1960). Briefly, blood samples were mixed with the anticoagulant Liquemin (0.5 mL per 10 mL blood). Each 2 mL were drawn up into syringes and kept inverted for blood cell sedimentation. The transparent layer down to 0.6 mL was discarded and the remainder (and 0.2 mL of the following) layer(s) was added to a culture flask containing a volume of 9 mL chromosome medium B (Seromed, with phytohaemagglutinin for mitosis stimulation). The incubation temperature was 37 °C.
The lymphocytes were exposed to single doses of beta-cyfluthrin (batch no.: 16001/85, purity: 98.8 %) in concentrations of 0-500-1000-5000 µg/mL. Two cultures per dose and donor were treated each in the presence and absence of metabolically activating S-9 mix. However, only one of both was used for chromosome evaluation the other serving as a spare. The treatment levels were chosen based on a pilot study with dosages ranging from 50 to 5000 µg/mL.
The test compound was dissolved in DMSO which also was used as negative (solvent) control in this experiment. A stability check in the solvent was performed before the experiment. Cyclophosphamide was applied as positive control in the activation test in a concentration of 15 µg/mL. Mitomycin C served as direct acting mutagen in the absence of S-9 mix at a treatment level of 0.15 µg/mL. Both controls were dissolved in Hank's salt solution.
The S-9 mix for metabolic activation was derived from the livers of at least six adult male Sprague-Dawley rats injected once intraperitoneally with Aroclor 1254 dissolved in corn oil at a dosage of 500 mg/kg bw 5 days prior to sacrifice (Ames et al., 1975). After sacrifice of the animals the livers were taken and kept at 4 °C. After washing with 0.15 M KCl solution, livers were homogenised in the

same solution. The supernatant was obtained after centrifugation (4°C, 9000 g, 10 min). The preparation of the S9 mix was in agreement with the publication of Ames et al. (1973). In short, the liver supernatant fluid was combined with an appropriate cofactor solution.
After cultivation of the cells for 48 h in the medium, the test material/positive and negative controls were added in portions of 0.1 mL (volume of test item solution was filled up with Hank's salt solution if quantities did not reach 0.1 mL). Furthermore, 0.1 mL S9- (for metabolic activation) mix or 0.1 mL of Hank's salt solution (test group without metabolic activation) was transferred to the cultures. The total volume of the cultures amounted to 10 mL. Exposure times were 2.5 h with S-9 mix and 24 h without S-9 mix. Cells under activation conditions were allowed to recover for 21.5 h until preparation. 3 h prior to harvest, colcemid (0.4 µg/mL) was added to arrest the cells in a metaphase-like stage of mitosis. All cell cultures were harvested 72 h following inoculation. Approximately two to three slides were prepared from each culture. After preparation of stained slides, the mitotic index was determined by counting 1000 cells per culture including spare cultures. Approximately 100 metaphases per culture were examined for chromosomal abnormalities. The clastogenic potential was evaluated by calculating the lesion/cell ratio and the percentages of aberrant cells including and excluding chromosome gaps. A light microscope (1000 x magnification) consisting of planapochromatic lenses was applied. Numerical aberration frequency was also recorded.
Evaluation criteria: The test is considered positive if there is a dose-dependent and statistically significant increase in the chromosome aberration rate over the negative control. A dose-dependent increase lacking statistical significance or a significantly elevated aberration frequency which is not concentration-related would be assessed equivocal. The acceptance of the test was guaranteed if both the positive and negative control led to results within the range of historical control ranges.

Results and discussions:

Table B.6.4-14: Mitotic index in human lymphocytes

Treatment groups	Concentration in µg/mL;	+/- S-9 mix	Nuclei evaluated	Absolute number of mitoses	Mitoses in % of negative control
DMSO	0	-	4000	155	100
Beta-cyfluthrin	500	-	4000	104**	67.1
Beta-cyfluthrin	1000	-	4000	70**	45.2
Beta-cyfluthrin	5000	-	4000	128*	82.6
Mitomycin C	0.15	-	4000	174	112.3
DMSO	0	+	4000	204	100
Beta-cyfluthrin	500	+	4000	105**	51.5
Beta-cyfluthrin	1000	+	4000	93**	45.6
Beta-cyfluthrin	5000	+	4000	131**	64.2
Cyclophosphamide	15	+	4000	165*	80.9

* = $p < 0.05$; ** = $p < 0.01$.

Mitotic index: In all beta-cyfluthrin treated cultures (with and without S-9 mix), the mitosis rate was reduced. In contrast to the statement of the study author, this decrease was not dose-related (Table B.6.4-14). In addition, precipitation of the test material in the culture medium was noted at 1000 and 5000 µg/mL. There was no reduction in the mitosis rate after treatment with mitomycin c whereas cyclophosphamide led to slight reduction.
Chromosome aberrations: There was no statistically significant increase in frequencies of structural or numerical chromosomal aberrations (aberrations including or excluding gaps, metaphases with exchanges) in any of the cell cultures treated without and with S-9 mix (Table B.6.4-15 and a 200 metaphases were evaluated, * $p \leq 0.05$, ** $p \leq 0.01$.

Table B.6.4-16). Treatment with both positive control substances gave the expected clear genotoxic responses which were statistically significant.

Table B.6.4-15: Results of cytogenetic study – Without S9 mix[a]

Dose [µg/ml]	Metaphases with aberrations incl. (excl.) gaps		Metaphases with exchanges		Polyploid cells in 400 evaluated metaphases	
	number	%	Number	%	number	%
0	7 (1)	3.5 (0.5)			4	1.0
500	8 (2)	4.0 (1.0)			2	0.5
1000	12 (3)	6.0 (1.5)			2	0.5
5000	4 (0)	2.0 (0)			2	0.5
Cyclophosphamide						
0.15	66** (35)**	33.0 (17.5)	4	2.0	1	0.3

[a] 200 metaphases were evaluated, * $p \leq 0.05$, ** $p \leq 0.01$.

Table B.6.4-16: Results of cytogenetic study – With S9 mix[a]

Dose [µg/ml]	Metaphases with aberrations incl. (excl.) gaps		Metaphases with exchanges		Polyploid cells in 400 evaluated metaphases	
	number	%	Number	%	number	%
0	13 (9)	6.5 (4.5)			0	0
500	11 (4)	5.5 (2.0)			0	0
1000	12 (3)	6.0 (1.5)			0	0
5000	10 (5)	5.0 (2.5)			0	0
Mitomycin c						
0.15	80** (51)**	40.0 (25.5)	20**	10	0	0

[a] 200 metaphases were evaluated, * $p \leq 0.05$, ** $p \leq 0.01$.

Conclusion:

Beta-cyfluthrin was found to be devoid of clastogenic potential in this study.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436**)** the study was considered to be acceptable. However, due to deviations from the current test guideline listed below the study is now considered to be no longer acceptable. It should be noted that the materials and methods section as well as the results and discussion section were revised.

The cytogenetic study was similar to the OECD-Guideline no. 473 (adopted May 26th, 1983). It remains unclear why the study was not based on this guideline as it already existed at the date of experimental work.

A preliminary test was conducted in order to select the concentrations for the study (50, 100, 500, 1000, 5000 µg/mL). According to the test guideline the highest dose should be based on a 50 % suppression of the mitotic activity. In case of precipitation, the substance should be tested up to the limit of solubility. In the preliminary test the mitotic index was - in comparison with the negative control - only 48.5 % at a dose of 500 µg/mL (without S9 mix) and 41.4 % at a dose of 5000 µg/mL (with S9 mix). Precipitation of the test compound was observed at 1000 µg/mL. Nevertheless, the applicants

chose a range of 500 to 5000 µg/mL for the main experiment (with/without S9 mix). This is neither in agreement with the guideline adopted in 1983 nor with the current guideline (adopted in September 26th, 2014). For this reason the study cannot be accepted. Furthermore, there are some other practical deviations from the test guideline: (1) Only one preparation time instead of test compound addition to cultures at various times following single harvest or single treatment following multiple harvest times; (2) only one sampling even though at least duplicate cultures should be used for each experimental point and (3) test substance not diluted in growth medium prior to treatment of cells. Moreover, there is a lack of documentation (e.g. CO_2 concentration not given in the test conditions).
From the perspective of the current guideline, the data required in the report is much larger (e.g. age of blood donors, methods used to determine pH, osmolality and precipitation, historical negative and positive control data). Furthermore, at least 300 metaphases per concentration and control should be scored according to current requirements and the exposure time in the presence of S9 mix is too short.
Under the conditions of the study and based on the information given in the report, no clastogenic potency of the test substance (with/without S9 mix) at concentration up to 5000 µg/ml was observed. However, the mitotic index was reduced in all dose groups in comparison to the negative control. The positive controls showed statistically significant clastogenic effects.

B.6.4.1.4 UDS test (primary rat hepatocytes)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.4.1 /03
Report:	Cifone, M. 1987, TOX9550278 Mutagenicity test on FCR 4545 technical in the rat primary hepatocyte unscheduled DNA synthesis assay. Report no.: R 4184, HLA study no.: 9778-0-447 (September 8, 1987), Sponsor Study no.: T 9024869; Hazleton Laboratories America, Kensington/MD, U.S.A.
Guideline(s):	Testing procedure was in compliance with OECD-Guideline no. 482 (1986).
Deviations:	Only mean values are reported.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(Dates of exp. work: March - April, 1987).

Materials and methods:

Unscheduled DNA synthesis (UDS) following treatment with beta-cyfluthrin (batch no.: 16001/85, purity: 99.5 %) was measured *in vitro* in primary rat hepatocytes (adult male Fischer 344 rat; source: Charles River Breeding Laboratories, incorporated; method: perfusion of liver with collagenase solution). Monolayer cultures were kept at 37 °C in a humidified atmosphere containing 5 % CO_2.
Williams' Medium E (WME) (supplemented with foetal bovine serum, L-glutamine, dexamethasone, penicillin, streptomycin sulfate, gentamycin) was used to establish the cell cultures (after establishment dexamethasone and serum components were removed).
The test material was dissolved in growth medium (WME, supplemented with 1 % foetal bovine serum) or, if it was incompletely soluble, in DMSO which served as negative solvent control, too. The positive control substance used in this experiment was 2-acetyl aminofluorene (2-AAF) at a concentration of 0.1 µg/mL medium (4.48×10^{-7} M).
The UDS test was initiated within 3 h after inoculation of culture dishes with approximately 0.5×10^6 viable cells (in WME , dexamethasone and 5 % serum) by replacing the culture medium with new WME containing 1 % foetal bovine serum, 1 µCi/mL ^{3}H-thymidine and the test material at the desired

concentration.
The cell cultures were exposed to the ingredient for 18 to 19 h. Beta-cyfluthrin was tested at 15 dose levels ranging from 0.025 µg/mL up to 1000 µg/mL. Since no signs of cytotoxicity were observed at lower concentrations, the eight upper doses ranging from 1.01 µg/mL to 1010 µg/mL (analytically determined) were selected for analysis of nuclear labelling and assessment of UDS (microscopically investigation, approx. 1500 x magnification). To determine the net nuclear grain count nuclear grains were counted and background count was subtracted.
Five cultures were treated per dose level. Three of them were used to prepare slides for autoradiography whereas the others were incubated for further 20 to 24 h to estimate cell survival. The net nuclear grain count was determined for 150 randomly selected cells per treatment level (50 on each of three coded coverslips derived from the three culture dishes).
Evaluation criteria: The test material was considered active in the UDS assay at concentrations causing:
(1) An increase in the mean net nuclear grain count to at least six grains per nucleus after subtraction of the concurrent negative control value, and/or (2) an increase in the percent of nuclei having six or more net grains to at least 10 % of the analysed population after subtraction of the concurrent negative control value, and/or (3) the percent of nuclei with twenty or more grains to reach or exceed 2 % of the analysed population.

Results and discussions:

A dose related increase in cytotoxicity was observed in this assay starting at 25.2 µg/mL and decreasing the survival rate to about 50 % at the highest dose tested. In addition, at the top dose of 1010 µg/ml, cells were obscured to precipitate. However, no significant increase in nuclear grain count or in the average percent number of nuclei with 6 or more grains over the vehicle control was noted at any of the dose levels assessed for UDS. No dose-dependent effect was apparent. Therefore, no evaluation criterion for a positive result was met (Table B.6.4-17). In contrast, the positive control substance induced a significant increase in all three evaluation parameters. Therefore, all three evaluation criteria for a positive outcome were met for 2-AAF. This indicates that the cell population is responsive and the methodology is adequate for the detection of UDS.

Table B.6.4-17: UDS Assay in primary rat hepatocytes

Dose [µg/ml]	UDS[a] grains/nucleus	Average[b] % nuclei with ≥ 6 grains	Average[b] % nuclei with ≥ 20 grains	Survival[c] (21h) [%]
1010	0.67	0.7	0.0	50.0
252	0.38	0.0	0.0	66.0
101	0.80	0.0	0.0	74.2
50.3	0.78	0.0	0.0	82.2
25.2	0.75	0.0	0.0	84.4
5.03	1.04	3.3	0.0	96.5
2.52	0.83	2.0	0.0	95.9
1.01	0.92	0.7	0.0	96.5
Solvent control (DMSO)				
1 %	0.57	1.3	0.0	100.0
Positive control (2-AAF)				
0.1	9.76	75.3	7.3	96.0

[a] UDS = Avergae of net nuclear grain counts on triplicate coverslips (150 total cells).
[b] Average values for triplicate coverslips.
[c] Survival = Number of viable cells per unit area relative to solvent control (*100).

Conclusion:

Beta-cyfluthrin is considered negative in this UDS-assay.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results section were revised.
The test was carried out in compliance with the demands of OECD-Guideline no. 482 (adopted October 23th, 1986). It remains unclear why the study report did not refer to this OECD-Guideline (as the guideline already existed at the date of experimental work).
Doses ranging from 1.01 µg/mL to 1010 µg/mL were tested. Only mean values were presented. Cytotoxic effects were indicated by the survival of cells (from 50 % after treatment with 1010 µg/mL test item to 96.5 % after treatment with 5.03 or 1.01 µg/mL test item). Precipitation of the test material was observed at 1010 µg/mL. Under the conditions of the study and based on the information given in the report, the test compound did not lead to significant increase in UDS in primary rat hepatocytes in comparison to the negative control. No dose-dependent response was apparent. However, treatment of cells with the positive control led to expected increases in nuclear labelling.

B.6.4.2 *In vivo* studies in somatic cells

B.6.4.2.1 Mouse micronucleus test

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.4 /03
Report:	[redacted] 1988, TOX9550279 FCR 4545 - Micronucleus test on the mouse to evaluate for clastogenic effects. Report No.: 16557 (March 24, 1988), Study No. T 8027117; [redacted]
Guideline(s):	The test was conducted in compliance with OECD-Guideline No. 474 (1983).
Deviations:	None. Deviation from recommendation in current guideline (OECD TG 2014) (only listed if relevant for acceptability decision): Only 1000 instead of 2000 (TG 1997) or 4000 (TG 2014) polychromated erythrocytes per animal evaluated.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered supplementary.

(Dates of exp. work: September - October, 1987).

Materials and methods:

Four groups of five young adult male and five virgin female mice (strain: Bor:NMRI [SPF Han]; source: [redacted]) each were administered beta-cyfluthrin (batch no. 16001/85, purity: 99.6 %) as a single dose of 80 mg/kg bw by oral gavage. The doses applied were chosen after performing a pilot test regarding mortality, in which male and female mice received orally different doses of the test compound. The control groups consisted of five male and female mice, too. The test substance was suspended in 0.5 % aqueous Cremophor emulsion. The stability of the test compound in the vehicle was checked before performing the experiment. Cyclophosphamide dissolved in deionised

water served as positive control compound and was applied by the same route at a concentration of 20 mg/kg bw. The negative control animals received orally 0.5 % aqueous cremophor emulsion. In all groups, the administered volume was 10 mL/kg bw.
For testing of chromosomal aberrations by counting micronuclei in polychromatic cells, animals belonging to three treatment groups were sacrificed at 24, 48 and 72 h following administration of the test substance at a dose of 80 mg/kg bw. The fourth group receiving a dose of 80 mg/kg bw beta-cyfluthrin was designated as replacement group. Positive and negative control mice were killed after 24 h only.
The smears were prepared as published elsewhere ((1) Schmid, The micronucleus test, Mutation Research 1975, (2) Schmid, Der Mikrokerntest, Deutsche Forschungsgemeinschaft, Mitteilung III, 1975). Briefly, a viscous cell suspension of bone marrow from one intact femur of each animal was placed on a slide, dried and stained. From these coded cell smears 1000 polychromatic erythrocytes were counted per animal using a light microscope (magnification about 1000). The incidence of cells with micronuclei was established. In addition, the ratio of polychromatic and normochromatic erythrocytes (e.g. in order to identify animals with pathological bone marrow depression that are excluded from the assessment) and the number of normochromatic cells with micronuclei (e.g. in order to identify animals subject to damage before conducting the test) were recorded.
Evaluation criteria: A significant increase in the number of polychromatic erythrocytes with micronuclei in comparison to the negative control at any of the test intervals may indicate a clastogenic potential of the compound.

Results and discussions:

Following oral administration of 80 mg/kg bw beta-cyfluthrin, the treated mice exhibited signs of toxicity for up to 24 h (e.g. apathy, uncoordinated movement, staggering gait and salivation). However, mortalities did not occur.
The results are presented in Table B.6.4-18. As there were no gender-specific differences, males and females were evaluated jointly.

Table B.6.4-18: Results of micronucleus test[a]

			Average no. of micronucleated cells per 1000 ± SD	
Test condition (dose in mg/kg bw)	**Time point of killing [h]**	**Average no. of normochromatic erythrocytes per 1000 polychromatic erythrocytes ± SD**	**Normochromatic erythrocytes**	**Polychromatic erythrocytes**
Negative control (-)	24	863 ± 252	0.9 ± 1.1	1.5 ± 1.2
Test compound (80)	24	736 ± 284	1.4 ± 1.5	1.1 ± 1.0
Test compound (80)	48	789 ± 269	1.2 ± 0.9	1.4 ± 1.1
Test compound (80)	72	747 ± 140	0.6 ± 0.8	1.2 ± 0.9
Positive control (20)	24	830 ± 289	0.9 ± 1.4	15.5 ± 7.4*

[a] 10000 polychromatic erythrocytes were evaluated.
* $p \leq 0.01$.

The ratio of polychromatic and normochromatic erythrocytes was not altered by treatment. There were no statistically significant or biologically relevant increases in the number of polychromatic erythrocytes with micronuclei at any of the test intervals. As expected in this case, the incidence of normochromatic cells with micronuclei was also not elevated. The positive control substance cyclophosphamide gave a clear genotoxic response regarding the number of micronucleated polychromatic cells. Again, the quantitative ratio between polychromatic and normochromatic cells was not altered.

Conclusion:

The micronucleus test in mouse bone marrow did not reveal evidence of a clastogenic potential of beta-cyfluthrin.

Re-evaluation by the RMS (2015):

The study is now considered to be supplementary. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered to be acceptable. The reasons for the supplementary status are deviations from the current guideline listed below. It should be noted that the materials and methods section as well as the results section were revised.
The micronucleus test was performed in mice and focussed on clastogenic effects as toxicological endpoint. The study met the criteria of OECD-Guideline no. 474 (adopted May 26th, 1983). It is unclear why the study was not based on this guideline (as it already existed at the date of experimental work).
Under the conditions of the study and based on the information given in the report, there was no statistically significant increase of micronucleated polychromatic erythrocytes at a dose level of 80 mg/kg bw in comparison to negative control. However, the number of polychromatic erythrocytes with micronuclei strongly increased after treatment with the positive control.
The current guideline demands the evaluation of at least 4000 polychromated erythrocytes per animal instead of 1000 (guideline from 1983) or 2000 (guideline from 1997).
Furthermore, the data requirements are more extensive in the current guideline (e.g. individual body weights at the start and end of the test, details of food and water quality, historical positive and negative control data).

The applicant disagreed with the acceptability status (supplemental). To their opinion the two deviations listed by the RMS are not critical enough to have an impact on the outcome of the study. However, due to the deviations listed above, the study is considered supplementary by the RMS. It is noted tha this decision has no impact on the overall conclusion that beta-cyfluthrin has no genotoxic potential.

B.6.4.3 *In vivo* studies in germ cells

No study was submitted since beta-cyfluthrin was negative in all *in vitro* and *in vivo* assays.

B.6.4.4 Further studies

The following publication and position paper were submitted:

Publication (Ila et al., 2008, ASB2014-7878) and position paper (Bayer CropScience, ASB2014-7879)

Data point:	KCA 5.4.1 /05
Publication	Ila *et al*., Mutation Research 656 (2008), 49-54: Genotoxic potential of cyfluthrin, ASB2014-7878
Position paper	Wason S., Bayer CropScience, BP 153, 06903 Sophia Antipolis Cedex, France (2013), 1-14: BES's position on the genotoxic potential of Cyfluthrin, ASB2014-7879
Noticeable issues:	*a) Salmonella/microsome assay* Less sensitivity (only two tester strains, *E. coli* WP2 or *S. typhimurium* TA102 for specific damages like oxidising mechanisms not included). No basis for dose selection presented (cytotoxicity or precipitation).

Type of values presented in the result table not specified (*e.g.* means, medians, SD, SEM).
Not mentioned which solvent was used as negative control.
No single data presented.

b) Chromosomal aberration/Sister chromatide exchange
Unclear why dose selection not based on precipitation or cytotoxicity.
Metabolic activation (S9 mix) not taken into account (from current guideline no. 473 only critical point if result without S9 is considered negative).
It seems that only one culture per donor was investigated.
Meaning of (error)bars presented in publication unclear (e.g. means, medians, SD, SEM).
Labelling "a" not specified in the representative figures.
Only slight increase of positive control after 48 h.
Results could be manipulated due to cytotoxic effects (especially after 48 h).
It is unclear whether the percentage of cells with structural chromosomal aberrations is presented in Fig. 1.
Dataset used for statistical analysis unclear.
No single data presented.

c) *In vitro* micronucleus formation test
Unclear why dose selection not based on precipitation or cytotoxicity.
Meaning of (error)bars presented in publication unclear (e.g. means, medians, SD, SEM).
It seems that only one culture per donor was investigated.
Labelling "a" not specified in the representative figures.
Metabolic activation (S9 mix) not taken into account (from current guideline no. 487 only critical point if result without S9 is considered negative).
It is unclear whether the percentage of cells with micronuclei is presented in Fig. 1.
Dataset used for statistical analysis unclear.
No single data presented.

d) *In vivo* tests (chromosomal aberration test and cytotoxicity)
Unclear which concentration of positive control was used.
Unit for doses "µg/ml" instead of "mg/kg" in Fig. 4 (not presented herein).
100 metaphases for each animal instead of at least 200 (current guideline) evaluated.
Meaning of (error)bars presented in publication not specified (e.g. means, medians, SD, SEM).
It is unclear whether the percentage of cells with chromosomal aberrations is presented in Fig. 2.
Results could be manipulated due to cytotoxic effects.
Dataset used for statistical analysis unclear.
No single data presented.

GLP: Not applicable.

Acceptability: The publication is considered not acceptable.

Materials and methods according to the publication:

Cyfluthrin (source: Bayer Turkey, CAS no: 68359-37-5, purity: 96.1 %) was used in different *in vitro* (Ames/microsome test, chromosomal aberration test, test for sister chromatide exchange and micronucleus formation) and *in vivo* tests (chromosomal aberration test and cytotoxicity) to evaluate the genotoxic potential. The *in vitro* and *in vivo* tests were performed with cultured human peripheral blood lymphocytes and rat (strain: *Rattus norvegicus* var. Albinos) bone-marrow cells, respectively.
T-tests were performed for significance analyses between the percentage of cells with chromosomal aberrations, mean SCE, percentage of binucleated cells with micronuclei, proliferation index, mitotic index, nuclear division index and numbers of revertants in the mutagenicity test in treated cultures and their controls.

a) Salmonella/microsome assay

Salmonella typhimurium tester strains TA98 (detecting frameshifts) and TA100 (detecting base-pair substitutions) were both from Dr. Rooney, USDA, U.S. The plate-incorporation test was conducted with/without S9 mix in order to investigate the mutagenic potential of cyfluthrin. Preparation of the S9 mix followed the instructions from Maron and Ames (Revised method for the Salmonella mutagenicity test, Mutation Research 113 [1983], 173-215). Briefly, S9 mixes were obtained from livers of male albino rats (previously treated with 80 mg/kg bw 3-methylcholanthrene in sunflower seed oil). Cofactors were combined added as commercially available S9 tablets.
Concentrations of test substance (dissolved in DMSO): 1000, 2000, 3000, 4000, 5000 µg/plate.
Positive controls (without S9 mix): 100 µg/plate 4-nitrophenylene diamine (TA98), 1 µg/plate sodium azide (TA100).
Positive control (with S9 mix): 20 µg/plate 2-aminofluorene (TA100 and TA98).
Negative control: 100 µL/plate.
A total of five plates for each experimental point were evaluated.

b) In vitro chromosomal aberration test and test for sister chromatide exchange

In general, the methods published from Evans (Human peripheral blood lymphocytes for the analysis of chromosome aberrations in mutagen tests, in B. J. Kilbey, M. Legator, W. Nichols, C. Ramel (Eds.), Handbook of Mutagenicity Test Procedure, 2nd ed., Elsevier Sci., BV, 1984, 405-427) and Perry and Thompson (The methodology of sister chromatide exchanges, in: B. J. Kilbey, M. Legator, W. Nichols, C. Ramel (Eds.), Handbook of Mutagenicity Test Procedure, 2nd ed., Elsevier Sci., Amsterdam, 1984, 495-529) were used.
Volumes of 200 µL blood samples were taken from two male und two female non-smokers (age between 22 and 25 years). Blood was added to 2.5 mL chromosome medium B (Biochrom) and (for SCE) further treated with 10 µg/mL bromodeoxyuridine (Sigma). Cultures were left for incubation at 37 °C for 72 h. The doses for cell treatment were derived from oral LD_{50} values in rats (869-1271 mg/kg bw): 500, 1000, 2000 µg/mL (24 h or 48 h). DMSO and mytomycin c served as negative or positive control, respectively. To arrest the mitosis, colchicine (0.06 µg/mL) was present for the final 2 h. Afterwards, cultures were centrifuged, treated with 0.4 % KCl, fixed and dried on slides. After staining structural and numerical aberrations were investigated (metaphase of 100 samples per donor, magnification: 1000). For SCE 25 cells were investigated (second metaphase, each donor). Furthermore, 100 cells from each donor were used for determination of the proliferation index.

c) In vitro micronucleus formation test

The test was performed according to the method published by Rothfuss et al. (Induced micronucleus frequencies in peripheral lymphocytes as a screening test of carriers of a BRCA1 mutation in breast cancer families, Cancer Research 60 [2000], 390-394). Blood was taken from healthy donors, added to 2.5 mL chromosome medium B and incubated at 37 °C for 68 h. Cells were treated with 500, 1000 or 2000 µg/mL cyfluthrin for 24 h or 48 h. DMSO served as solvent control. Mitomycin c was selected as positive control at a concentration of 0.25 µg/mL. To block cytokinesis, 6 µg/mL cytochlasin B (Sigma) was added 44 h after initiating the cultures. Harvest of cultures was conducted 14 h after-

wards. Then, cells were treated with 0.4 % KCl and fixed. Slides were dried and stained. For the evaluation of micronucleus formation 1000 binucleated lymphocytes were scored per donor (4000 per concentration). The nuclear division index was determined from 500 cells.

d) *In vivo tests (chromosomal aberration test and cytotoxicity)*

Each two rats per gender were used for the control and the treatment groups. Doses of 250, 500 or 1000 mg/kg bw cyfluthrin were administered orally or by intraperitoneal injection. DMSO and urethane served as negative or positive control, respectively. Animals were sacrificed after 12 or 24 h.
A dose of 3 mg/kg bw colchicine was injected intraperitoneally 2 h before sacrifice of the animals in order to arrest mitosis. Animals were killed by cervical dislocation. Then, bone marrow was obtained from femurs by aspiration in 0.9 % NaCl balanced solution. The bone-marrow pellet after centrifugation was resuspended in 0.4 % KCl. Cells were fixed after further centrifugation, dried on glass slides, stained and mounted with entellan. An amount of 100 metaphases per animal (totally 400) was investigated (magnification: 1000). Mitotic indices were derived from evaluating 300 cells per animal.

Results according to the publication:

a) Salmonella/microsome assay

No (dose-related) significant ($p > 0.05$) increase in revertants per plate was observed in both tester strains (with/without S9 mix). The positive controls led to strong increase in the number of revertants/plate.

b) In vitro chromosomal aberration test and test for sister chromatide exchange

The frequency of chromosomal aberrations after treatment with 500, 1000 or 2000 µg/mL cyfluthrin for 24 h was not statistically significant ($p > 0.05$) increased in comparison with the negative control. However, statistically significant increases were observed after 48 h (1000 or 2000 µg/mL) ($p < 0.05$, Figure B.6.4-1). The effects were not dose-related. The number of chromosomal aberrations in the positive control was only slight increased after 48 h in contrast to the results observed after 24 h. There was no statistically significant increased level of structural chromosomal aberrations per cell compared to the control.

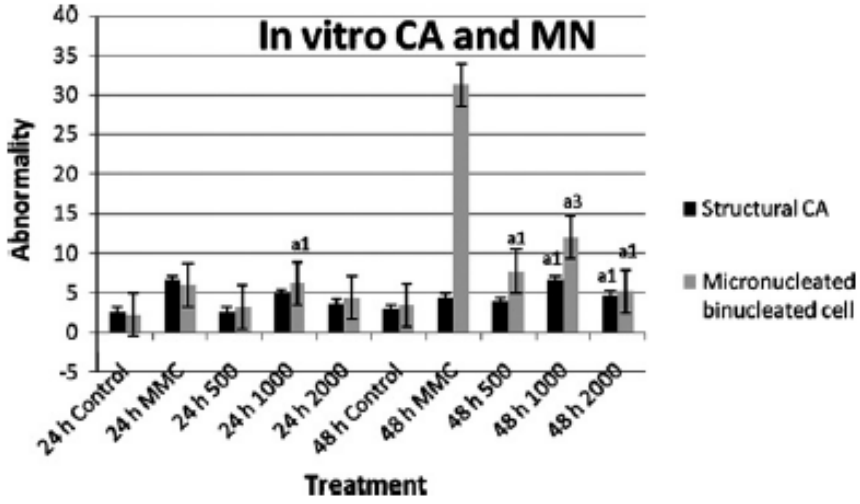


Figure B.6.4-1: Frequency of chromosome aberrations and micronucleus formation. Graphic representation of frequencies of both structural CA and MN formation in cultured human lymphocytes.

The frequency of sister chromatide exchanges after treatment with the test compound did not increase in a statistically significant manner ($p > 0.05$) after 24 or 48 h. Strong effects were observed in the positive control. The proliferation index was statistically significant reduced at all three doses after 48 h.

c) In vitro micronucleus formation test

The frequency of micronucleated cells was statistically significant increased (at least $p < 0.05$) at a concentration of 1000 µg/mL after 24 h and at all concentrations after 48 h in comparison with the solvent control (not dose-dependent) (Figure B.6.4-1, see above). Frequencies of micronucleus formation were also increased in the positive control. There was a statistically significant decrease of the nuclear division index when compared with the solvent control.

d) *In vivo* tests (chromosomal aberration test and cytotoxicity)

Cyfluthrin increased the frequency of chromosome aberrations in all treatment groups, via both administration routes, during both treatment periods in a statistically significant manner (at least $p < 0.05$) (Figure B.6.4-2). Chromosome aberrations increased also in the positive control.

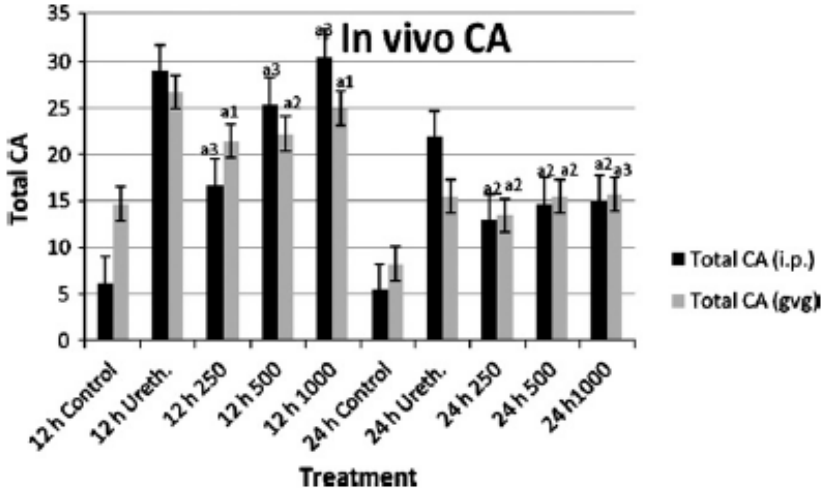


Figure B.6.4-2: Chromosome aberrations in bone-marrow cells of rats treated with cyfluthrin. Graphical presentation of relationship between intraperitoneal treatment and oral gavage treatment.

However, the numerical chromosome aberrations/cell findings were not statistically significant, Formation of chromosomal aberrations did not occur dose-related. The mitotic index decreased statistically significant after oral (24 h) administration of the test compound.

Conclusion by Ila et al., 2008, ASB2014-7878**:**

a) Salmonella/microsome assay

Cyfluthrin is not mutagenic in TA98 and TA100 (with or without metabolic activation).

b) In vitro chromosomal aberration test and test for sister chromatide exchange

Cyfluthrin did not lead to SCE formation but to induction of chromosome aberrations in cultured human blood lymphocytes possibly due to clastogenic potential (two highest concentrations after 48 h). Cytotoxicity (48 h after treatment) was derived from the statistically significant reduction of the proliferation index.

c) In vitro micronucleus formation test

Cyfluthrin led to micronucleus formation possibly due to clastogenic effects. The reduced nuclear division index points to cytotoxicity.

d) In vivo tests (chromosomal aberration test and cytotoxicity)

Chromosomal aberrations were induced by cyfluthrin. This finding points to clastogenic potential. Cytotoxic activity was inferred from decreased mitotic indices.

Conclusion by position paper (S. Wason from Bayer CropScience, 2013, ASB2014-7879**):**

It was concluded that the study should not be taken into account to evaluate the genotoxic potential of cyfluthrin. The following arguments led to this conclusion: (1) incompleteness of the data presented (no historical control data); (2) no guideline compliance; (3) non-GLP-conformity; (4) questionable study design and (5) doubtful reliability and robustness of data. It was also mentioned that all other *in vitro* and *in vivo* genotoxicity studies with cyfluthrin (performed at Bayer CropScience) were negative and no tumour formation was observed in long-term studies with mice and rat.

Conclusion by Prof. D. Kirkland (expert statement, ASB2014-7879**):**

From the data of the bacterial mutagenicity test it was suggested that cyfluthrin is not mutagenic. However, the limited sensitivity of the mutagenicity test was criticised as not all required bacterial strains were involved in the study.
Cyfluthrin did not increase the frequency of sister chromatide exchanges *in vitro*. However, it was stated that the data should be omitted anyway for assessment of genotoxicity as from the today's perspective sister chromatide exchanges are no conventional endpoint for evaluating the genotoxic potential of a substance.
Regarding the *in vitro* micronucleus test and the chromosomal aberration tests performed *in vitro* and *in vivo* it was suggested that the test compound could be clastogenic. However, it was emphasised that some limitations in the validity make it difficult to draw a final conclusion whether the effects observed are true or provoked by the extreme conditions. In this context, poor presentation of data (e.g. no units on Y-axis), inadequate and inconsistent responses of the positive control, cytotoxic test concentrations (e.g. impact on dose-response or indirect induction of chromosomal aberrations or micronuclei) and irritant properties of the vehicle DMSO *in vivo* are listed.

Conclusion by the RMS (2015):

a) Ames/microsome test

The mutagenic potential of cyfluthrin was investigated in the Ames/microsome test.
Under the conditions of the study and based on the information given in the report, cyfluthrin is not mutagenic in the bacterial strains TA98 and TA100. However, from the today's perspective there are some deviations in the study design that limit the informative value: As only the two bacterial tester strains TA100 and TA98 were used, specific damages - originated for example from oxidising mechanisms – are not taken into account. To circumvent this problem the current guideline involves additional strains like *E. coli* WP2 and TA102 in the study. The dose selection for the mutagenicity testing remains unclear. It is recommended to base the dose regimen on precipitation of the test compound or cytotoxicity in order to prevent deviations from actual dose or genotoxic effects. Furthermore, the solvent for cyfluthrin is not named in the study. The type of values presented in the result table are not specified (e.g. mean, median, SD, SEM). No single data are presented.

b) Chromosome aberration/Sister chromatide exchange

Cyfluthrin was tested concerning its ability to induce sister chromatide exchanges and chromosomal aberrations *in vitro*.
Under the conditions of the study and based on the information given in the report, the test compound

did not lead to the formation of sister chromatide exchanges, but to chromosomal aberrations in cultured human blood lymphocytes. However, from the today's perspective some deviations in the study design may affect the robustness, reliability and informative value of the data: It is unclear why the dose selection was not based on precipitation of the test compound or cytotoxicity. For this reason it remains unclear whether there are deviations from actual dose or genotoxic effects. The response of the positive control for chromosomal aberrations was only weak after 48 h. Cytotoxic activity could be the reason for deviations from dose-related effects. Concerning the sister chromatide exchange it is striking that metabolic activation (S9 mix) was not included. From the current guideline for chromosomal aberration testing an additional investigation with S9 mix becomes necessary if the study outcome is negative. It seems that only one culture per donor was investigated. The (error)bars presented in the diagram as well as the labellings "a" are not specified (e.g. mean, median, SD, SEM) (see Figure B.6.4-1). No single data are presented. The poor legend in the figure makes it difficult to relate the results and conclusions in the text to the bars in the figure (see Figure B.6.4-1). It is for example unclear whether the percentage of cells with structural chromosomal aberrations is presented in Figure B.6.4-1. Furthermore, the dataset used for statistical analysis is not provided. It should be mentioned that the OECD-Guideline for sister chromatide exchange was deleted this year. That is why this experiment is of lower relevance for evaluating the genotoxic impact of cyfluthrin.

c) *In vitro micronucleus formation test*

Micronucleus formation by cyfluthrin was also studied in cultured human blood lymphocytes.
Under the conditions of the study and based on the information given in the report, micronuclei were induced by the test compound. However, from the today's perspective some deviations in the study design may affect the robustness, reliability and informative value of the data: In the study it is unclear why dose selection was not based on precipitation of the test compound or cytotoxicity. Even though the NDI was not strongly increased, the PI and MI values (especially after 48 h) point to cytotoxic effects that may be indirectly responsible for the increased MN frequencies. The effects observed were not dose-related. As precipitation of the test compound was not tested, deviations from actual dose shall be taken into account. The current guideline for micronucleus testing does not require endpoints with metabolic activation (S9 mix) in case of a positive outcome (as reported in this study). It seems that only one culture per donor was investigated. Again the meaning of (error)bars presented in the diagram as well as the labellings "a" are not explained (e.g. mean, median, SD, SEM) (see Figure B.6.4-1). No single data are reported. The poor legend in the figure hampers a relation of the results and conclusions in the text to the bars in the figure (see Figure B.6.4-1). It is for example unclear whether the percentage of cells with micronuclei is presented in Figure B.6.4-1. Furthermore, the dataset used for statistical analysis is not specified.

d) In vivo tests (chromosomal aberration test and cytotoxicity)

The potential to induce chromosomal aberrations was also studied in rat bone-marrow cells in order to evaluate the genotoxicity *in vivo*. Under the conditions of the study and based on the information given in the report, chromosomal aberrations were detected. However, from the today's perspective some deviations in the study design may affect the robustness, reliability and informative value of the data: The doses of the test compound administered to the animals seem quite high. Information about clinical signs of the animals or other suffering from these high doses is not given. The concentration of the positive control is not given. The units in the legend of figure 4 (not shown herein) differ from the units mentioned in the text ("µg/mL" instead of "mg/kg"). Some values of the mitotic index in the rat bone-marrow cells point to about 50 % cytotoxicity. This could have had an impact on the (dose-related) genotoxic effects. Only 100 metaphases (instead of 200 according to the current guideline) per animal were investigated. Again the meaning of (error)bars presented in Figure B.6.4-2 as well as the labellings "a" are not explained (e.g. mean, median, SD, SEM). No single data are reported. The poor legend in Figure B.6.4-2 makes it difficult to relate the results and conclusions in the text to the bars in Figure B.6.4-2. It is for example unclear whether the percentage of cells with chromosomal aberrations is presented in Figure B.6.4-2. Furthermore, the dataset used for statistical analysis is not specified.

In summary, the publication from Ila et al. (2008, ASB2014-7878) comprises several genotoxicity studies with a questionable study design and side effects like cytotoxicity that may affect the robustness, reliability and informative value of the data. Furthermore, poor presentation of data makes it difficult to follow and understand the results and conclusions of the authors.
For this reason, the publication is considered not acceptable.

Further studies available to RMS:

The studies listed below were not submitted by the applicant for renewal of approval. However, the studies are available to RMS (e.g. from other applications).
Studies with preparations are not included as they are less relevant for the evaluation of the active ingredient.
The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are summarised hereafter (Table B.6.4-19 and Table B.6.4-20). Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table B.6.4-19: Summary of *in vitro* studies – cyfluthrin

Test system	Test object	Concentration, (vehicle)	Result	Comment	Reference
Ames test	Bacterial strains: TA1513, TA100, TA1537, TA98	0, 20, 100, 500, 2500, 12500 µg/plate (DMSO)	negative +/- S9 mix	- less sensitivity as only four instead of five bacterial strains were applied (*E. coli* WP2 or *S. typhimurium* TA102 not included) (TG 471, 1997) - beginning at 2500 µg/plate precipitation - doses up to 24000 µg/plate not cytotoxic - no spread or significance test given - no single data	Herbold, 1980 (9273) TOX9401890
Ames test	Bacterial strains : E. coli B/r WP2 try- hcr-, TA1535, TA1537, TA1538, TA98, TA100	0, 5, 10, 100, 500, 1000, 5000 µg/plate (DMSO)	negative +/- S9 mix	- only duplicates instead of triplicates (TG 471, 1983 and 1997)	Nagane, Hatanaka, Iyatomi, 1982 (MO-01-004657) TOX9401894
Ames test	Bacterial strains : E. coli WP2 hcr and *Salmonella* TA1535, TA1537, TA1538, TA98, TA100	0, 50,100,500,1000, 5000, 10000, 25000 µg/plate (DMSO)	negative +/- S9 mix	- only duplicates instead of triplicates (TG 471, 1983 and 1997) - 2-aminoanthracen is solely used as positive control with S9 mix (TG 471, 1997) - no batch given	Ohta and Moriya, 1982 (MO-01-004654) TOX9401895

HGPRT	CHO cells	0, 3, 5, 7, 9, 10 µl/ml (DMSO)	negative +/- S9 mix	- not tested up to obvious cytotoxicity	Yang, 1985 (MO-01-003008) TOX9401899
cytogenetic study (clastogenic effects)	Chinese hamster lung cells	0, $3 \cdot 10^{-5}$, $1 \cdot 10^{-4}$, $3.3 \cdot 10^{-4}$, $1 \cdot 10^{-3}$, $3.3 \cdot 10^{-3}$ M (DMSO)	negative +/- S9 mix	- turbidity at three highest concentrations (+/- S9 mix, 50 % suppression of cell growth at highest concentration (only + S9 mix) - test substance not diluted in growth medium (473, 1984) - no short-time exposure without S9 mix (TG 473, 2014) - only 200 metaphases per experimental point evaluated instead of 300 (TG 473, 2014)	Sasaki, Imanishi, Watanabe, Ohta, 1986 (MO-01-004656) TOX9401901

For FCR 4545 no further studies are available.

Other *in vitro* studies with cyfluthrin:

In this section studies are summarised which are used less commonly for the evaluation of the genotoxic potential.
Cyfluthrin was negative in the rat hepatocyte UDS assay at a dose ranging from 17-5000 µg/ml (Curren, 1985, MO-01-003009, TOX9401900). Furthermore, the test substance led to negative results +/- S9 mix in the Pol A^1-test using *E. coli* pol A^+ and pol A^- at doses from 62.5 to 1000 µg/plate (Herbold, 1981, 10450, TOX9401893). No DNA-damaging effects of FCR 1272 were detected in the rec-assay with strains of *Bacillus subtilis* at 200 µg/disc (Nagane, Hatanaka, Iyatomi, 1982, MO-01-004657, TOX9401894) or from 100-10000 µg/disc without S9 mix (Moriya and Ohta, 1982, MO-01-004654, TOX9401895). Tests for Reverse mutation induction, mitotic crossing over or gene conversion were performed in *Saccharomyces cerevisiae* by Brusick (1982, TOX9401896 and TOX9401897). There was no reverse mutation induction +/- S9 mix at doses from 312.5 to 10000 µg/ml (Brusick, 1982, R2248, TOX9401896), neither reverse mutation induction, mitotic crossing over nor gene conversion from doses ranging from 0.625 to 10 mg/ml +/- S9 mix (Brusick 1982, R2249, TOX9401897).

Table B.6.4-20: Summary of *in vivo* studies – cyfluthrin

Test system	Animal (sex, route, vehicle)	Concentration	Result	Comment	Reference
Micronucleus test	mouse (both sexes, oral, PEG 400)	2*7.5 mg/kg (24 h intervall) 2*15 mg/kg bw (24 h intervall)	negative	- positive control in water was given via i.p. injection - dose selection based on preliminary test (only weak symptoms at 15 mg/kg bw) - only two dose levels instead of three (TG 474, 2014) - only one instead of three sampling time	[redacted] 1980 (9435) TOX9401891

				points (474, TG 1983) - one sampling in case of two treatments, but sampling 18-24 h (TG 474, 2014) following final treatment (here 6 h) - 1000 instead of 4000 immature erythrocytes per animal scored (TG 474, 2014)	

For FCR 4545 no further studies are available.

Other *in vivo* studies with cyfluthrin:

A dominant lethal test ([redacted], 1981, 9678, TOX9401892) was performed but should be excluded from the evaluation as no positive control was used.

B.6.5 Long-term toxicity and carcinogenicity

No new data on chronic toxicity and carcinogenicity have been generated since Annex-I inclusion of cyfluthrin/beta-cyfluthrin and the publication of the addendum 1 (2002). All studies were conducted with cyfluthrin and have been previously submitted. For the renewal process they were again evaluated.

A literature research for the Renewal Assessment Report (RAR) including publications from the last 10 years was performed by the RMS. For the chapter long-term toxicity and carcinogenicity no publications were found.

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

B.6.5.1 Rat

Data point:	KCA 5.5
Report:	[redacted], 1983, TOX9401904: FCR 1272 (Cyfluthrin, the active ingredient of Baythroid) - chronic toxicity study on rats (2-year feeding experiment). Report no.: 11949 (report), 11949A (addendum), (July 19, 1983); [redacted]
Guideline(s):	The test was run principally according to OECD-Guideline no. 453 which complies to Directive 87/302/EEC, part B.
Deviations:	Deviations from the mentioned guideline: The purity of the test substance was not given. Interim kill and satellite animals did not underwent the same observations, including body weight, food/water consumption, haematological and clinical biochemistry measurements and pathological investigations as the animals of the carcinogenicity study, ophthalmological examinations were not performed, neurotoxicity tests were not performed, Water consumption was not measured, Clinical laboratory examinations (Hematology, clinical chemistry) was not performed after three months, the amount of albumin was only determined by protein electrophoresis after 12 months. Coagulating-, Harderian-, lacrimal-, mammary gland, epididymis, skin, vagina, bone marrow were not preserved, weights of brain, epididymis, thyroid gland and uterus were not taken and preserved. Additional investigations: The liver function (N-demethylase, O-demethylase, cytochrome P-450, alkaline phosphatase) and 12 and 24 months after the start of the study the concentration of fluoride in bones and teeth were determined.
GLP:	When the study was performed, GLP was not compulsory. However main activities were performed according to the OECD principles of GLP (declaration of testing facility in tier I).
Acceptability:	Not acceptable.

(Dates of exp. work: September 1980 - September 1982).

Materials and methods:

Groups of 65 male and 65 female Wistar rats (Bor:WISW (SPFCpb); source: Winkelmann, Borchen, Germany) received cyfluthrin via the feed for 2 years in concentrations of 0, 50, 150 and 450 ppm, corresponding to 2.02, 6.19 or 19.20 mg/kg bw/d for males and 2.71, 8.15 or 25.47 mg/kg bw/d for females. The test compound was a composite sample of 5 different batches, available as a pre-mix concentrate with Wessalon S (from August 1981 onward with 1 % peanut oil) with a cyfluthrin content of approx. 50 %. The purity of individual batches was not given.
Five of the animals per sex and dose were used to determine microsomal enzyme activities after the first week of the study. Ten animals per sex and dose were used for the interim autopsy after the first year of treatment.
Statistical methods: The values of the treated groups were compared with the control values by the Wilcoxon-Mann-Whitney U-test. The mortality rates were compared by Fischer´s exact test.

Results and discussions:

Findings:
In appearance, behaviour, food consumption and survival rate the animals treated with cyfluthrin did not differ from the controls. The dose of 150 ppm produced a transient retardation of growth, while at 450 ppm growth was clearly retarded for the entire experimental period (see Table B.6.5-1).

Table B.6.5-1: Mortality and body weight

Dose [ppm] **Sex**	**0** **M/F**	**50** **M/F**	**150** **M/F**	**450** **M/F**
Mortality (24 mo) [%]	12/14	8/10	4/10	18/18
Body weight (12 mo) [g] + SD [g] Signi	435/234 19/15	418/235 39/23	385/247 25/26 **/	371/208 27/11 **/*
Body weight (24 mo) [g] + SD [g] Signi	418/265 46/26	408/266 37/29	410/252 48/33 /*	382/237 34/25 **/**

* = p <0.05; ** p <0.01.

Clinical laboratory tests: After 6 months, the leukocyte counts were significantly increased at 450 ppm in males and females, and at 150 ppm in females. After 18 months it was significantly lower in females at 450 ppm. After 24 months, it was decreased in males at all dosage groups (see Table B.6.5-2). There was no clear dose-response relationship for this parameter. Significant increases over control in the blood glucose concentration were determined after 18 months for the male rats at all dosage levels. This parameter was not increased statistically after 12 and 24 months for males. Furthermore, the blood glucose concentration was not increased over the control for the females after 18 months. In addition some parameters differed significantly from the respective control group in males and females. Since changes were observed only at isolated examination times and/or only in one sex and/or they did not occur dose-related no toxicological relevance is attributed to these findings.
The fluoride content in teeth and bones of treated animals was similar to those of control values at month 12 of the study. Increased fluoride levels were noted after 24 months in the teeth and bones of males receiving the high dose, and in the bones of males receiving the mid-dose and females receiving the high-dose level (10 to 16 % over control).
Enzyme induction assay: No differences were noted in N- or O-demethylase activities or cytochrome P450 levels in treated animals when compared to control values, except for a significant increase in N-demethylase activity (N-d-lase) in females receiving the high-dose.

Table B.6.5-2: Clinical laboratory tests

Dose [ppm] Sex	0 M/F	50 M/F	150 M/F	450 M/F
Leucocytes (6 mo) [giga/L] + SD [giga/L] Signi	9.1/6.8 1.1/1.0	8.9/7.7 1.6/1.8	9.3/8.0 1.1/1.5 / *	11.8/7.9 2.9/0.9 * / *
Leukocytes (18 mo) [giga/L] + SD [giga/L] Signi	6.5/4.4 1.6/0.7	5.7/4.3 1.2/0.7	5.5/5.3 0.9/3.4 /	5.5/3.7 1.2/0.7 / *
Leukocytes (24 mo) [giga/L] + SD [giga/L] Signi	7.3/5.7 1.0/14.4	5.6/4.4 0.9/0.6 ** /	5.8/5.3 0.8/1.9 * / .	5.8/4.8 1.7/0.9 * /
Glucose (18 mo) [mmol/L] + SD [mmol/L] Signi	4.46/4.95 0.6/0.7	5.18/4.78 0.5/0.6 * /	5.25/5.28 0.6/0.5 * /	5.42/5.30 0.3/0.5 * /
N-d-lase (7 d) [nmol/g/min] + SD [nmol/g/min] Signi	108/59 12/6	108/69 13/14	109/71 33/9	135/104 37/14 / *

* p <0.05, ** p <0.01., 1 one uncommon value at animal-no 87 with 18,1;
N-d-lase = N-demethylase activity.

Pathological examinations: The absolute liver weight was significantly decreased after 12 and 24 months (

Table B.6.5-3 and Table B.6.5-4). The relative weight of the adrenals was increased after 24 months in females in the highest dose group.

Table B.6.5-3: Organ weights

Dose [ppm] Sex	0 M/F	50 M/F	150 M/F	450 M/F
Liver (abs.,12 mo) [mg] SD [mg] Signi	15610/8361 1921/851	14828/7311 1648/1108	13245/7506 1775/1053	12900/6781 376/776 **/**
Liver (abs.,24 mo) [mg] + SD [mg] Signi	14192/9332 1839/1060	14607/9156 1989/1196	14242/8508 2152/1117 /**	12975/ 8330 1699/1158 **/**
Adrenals (rel./24 mo)[%] + SD [%] Signi	10/25 2/12	11/24 2/7	11/25 3/7 */	16/29 26/14 / **

* = p <0.05; ** = p <0.01.

The examined organs of the rats of all dose groups showed spontaneous inflammatory or degenerative changes. In female rats an increase of adrenal cortical hyperplastic nodules and of ovarial stromal hyperplasia was found. The adrenal glands of males showed an increased incidence of medullary hyperplasia.

Table B.6.5-4: Histopathology, non-neoplastic lesions

Dose [ppm] Sex	0 M/F	50 M/F	150 M/F	450 M/F
Ovaries (no) -Stromal hyperplasia	/50 /3	/50 /6	/50 /9	/50 /9
Adrenals (no) -Cortic. hyp. nodule -Medull. hyperplasia	48/50 10/4 4/5	48/49 21/9 8/9	49/50 14/11 8/1	50/49 20/18 14/4

(no) = number of organs examined

In every group the range of tumours was normal for rats of the given age and conformed to the relevant experience with this strain. A slight increase in the combined incidence of medullary hyperplasia and pheochromocytomas in the adrenals of male rats was observed. No evidence of oncogenicity of the substance at any dose could be derived from the type, localisation, incidence and latency of neoplasias found.

Table B.6.5-5: Histopathology, neoplastic lesions

Dose [ppm] Sex	0 M/F	50 M/F	150 M/F	450 M/F
Adrenals [no#] -cortical carcinoma (m) -pheochromocytoma (b/m)	48/50 0/1 4/0	48/49 0/0 3/2	49/50 0/0 5/1	50/49 0/0 6/1
Bones [no#] -osteochondroma (b) -fibrosarcoma (m)	49/50 0/0 1/0	50/50 0/0 0/0	49/50 0/0 0/0	50/49 1/0 0/0
Brain [no#] -meningioma (b) -astrocytoma (b)	49/50 0/0 0/2	50/50 1/0 1/0	49/50 0/0 0/1	50/49 0/0 0/0
Cutis and subcutis [no#] -adenoma (b) -basal cell carcinoma (m) -squam. cell carcinoma (m) -lipoma (b) -malign. neurilemmoma (m) -fibrosarcoma (m) -fibroma (b)	1/4 0/0 0/0 0/0 0/0 0/0 0/0 1/0	2/10 1/0 0/1 0/1 1/0 0/1 0/2 0/0	0/4 0/0 0/0 0/1 0/0 0/0 0/1 0/0	5/4 0/0 0/0 0/0 0/1 0/0 2/0 1/0
Heart [no#] -aortic body tumor (b) -endocardial tumor (b) -endocardial sarcoma (m)	49/50 0/0 1/0 0/0	50/50 1/0 1/0 0/0	49/50 0/0 0/0 0/0	50/49 0/0 0/0 0/1
Kidneys [no#] -adenoma (b) -lipomatous tumor (b)	49/50 0/0 0/0	49/50 0/0 1/0	49/50 1/0 1/1	50/49 0/0 0/0
Liver [no#] -carcinoma (m)	49/50 0/0	50/50 0/0	49/50 0/0	50/49 1/0
Lymph nodes [no#] -hemangioma (b)	49/50 0/0	49/47 1/0	46/48 0/0	50/49 0/0
Mammary glands [no#] -carcinoma (m) -fibroadenoma (b)	0/5 0/1 0/5	0/5 0/1 0/3	1/4 0/0 0/3	0/5 0/0 0/3
Ovaries [no#] -gran. theca cell	0/50 0/3	0/50 0/0	0/49 0/2	0/49 0/2

Pancreas [no#] -islet cell tumor (b) -exocrine adenoma (b)	48/50 0/0 0/0	48/50 0/0 1/0	49/49 2/0 0/0	50/49 0/0 0/0
Parathyroids [no#] -adenoma (b)	15/14 2/0	6/14 1/0	6/13 0/1	14/15 0/0
Pituitary [no#] -adenoma (b)	47/49 10/14	49/50 12/23	47/48 19/12	47/48 7/12
Reticuloend. tissue [no#] -malignant lymphoma (m) -malignant histiocytoma(m)	49/50 0/0 0/0	50/50 0/0 1/1	49/50 0/0 0/0	50/49 1/0 2/0
Spleen [no#] -hemangioma (b)	49/50 0/0	48/50 0/0	49/50 1/0	50/49 0/0

Table B.6.5-6 (cont.): Histopathology, neoplastic lesions

Dose [ppm] Sex	**0 M/F**	**50 M/F**	**150 M/F**	**450 M/F**
Testes [no#] -Leydig's cell tumor (b) -mesothelioma (b)	49/0 3/0 2/0	49/0 5/0 0/0	49/0 5/0 0/0	50/0 4/0 4/0
Thymus [no#] -squam. cell carcinoma (m)	0/0 0/0	0/0 0/0	0/1 0/1	0/0 0/0
Thyroid [no#] -adenoma (b) -carcinoma (m)	49/49 4/2 0/0	48/48 2/1 2/0	47/49 2/1 0/3	48/47 1/0 1/0
Urinary bladder [no#] -papilloma (b)	48/50 0/0	48/49 0/0	49/48 0/0	50/49 0/3
Uterus [no#] -adenocarcinoma (m) -polyp (b)	0/50 0/5 0/14	0/50 0/4 0/7	0/50 0/4 0/20	0/49 0/3 0/17

[no#] no. of rats examined; (b) benign; (m) malignant

In the addendum Suberg and Löser, 1983, TOX9401904 (July 19, 1983) data not reported in the original report were given, i.e. results of clinical observations of individual animals, information about the test substance and about analyses of the mixtures of the test substance (stability, homogeneity, concentration in the mixture), supplementary information on histopathological investigations.

Conclusion:

The NOAEL was determined at 50 ppm, equal to 2.02 mg/kg bw/d in males and equal to 2.71 mg/kg bw/d in females. It was based on a retardation in growth of rats at the dose of 150 ppm and above. A NOAEL was established, since some parameters were changed statistically significant from the lowest dose onward (e.g. leukocyte count, blood glucose). However these changes are not considered as toxicological significant by the RMS.
This study served as the basis for calculation of the proposed ADI for cyfluthrin as well as for beta-cyfluthrin.

Re-evaluation by the RMS (2015):

The assessment of the study has changed from the initial evaluation in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), when the study was considered acceptable. At present the study cannot be considered acceptable anymore. The main reason is the missing information on the purity of the test substance (cyfluthrin) and the missing of a clear distinction between the chronic phase (after 12 months) and the long-term phase (after 24 months). The results of examinations after

12 and 24 months are not reported separately. In addition, a variety of examinations were not performed at all (see deviations) in the course of the entire study. The information/results in the addendum are considered. The study was formerly used for the derivation of the ADI-value. The NOAEL was set at 2.02 mg/kg bw/d for males and 2.71 mg/kg bw/d for females based on slightly reduced body weight after 12 and 24 months in males and females at 150 ppm (6.19/8.15 mg/kg bw/d) and after 24 months in both gender at 450 ppm (19.20/25.47 mg/kg bw/d).

B.6.5.2 Mouse

Data point:	KCA 5.5
Report:	[redacted], 1983, TOX9401905 Suberg, H. and Löser, E.: FCR 1272 - Chronic toxicological study on mice (feeding study over 23 months). Report no.: 12035 (August 24, 1983); [redacted] T0000271: Addendum to Report No. 12035 A (September 22, 1994); [redacted]
Guideline(s):	The test was only partly run according to OECD-Guideline no. 453 which complies to Directive 87/302/EEC, part B.
Deviations:	The purity of the test substance was not given; clinical laboratory examinations (Hematology, clinical chemistry) were not performed after three months; ophthalmological and neurotoxicity tests were not performed; water consumption was not measured, blood clotting parameters were not determined; very limited parameters in clinical chemistry were determined (i.e. no glucose, urea, total protein and albumin etc.); no interim sacrifice was performed; overview on necropsy findings are missing; adrenals, brain, epididymides, testes , thyroid, uterus were not weighed; cervix, epididymis, lacrimal gland, thymus, vagina, bone marrow were not taken and preserved; the histopathological investigations did not include parathyroid and thymus. Additional investigation: The concentration of fluoride in bones and teeth was estimated at termination of study.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Not acceptable

(Dates of exp. work: November 1980 - October 1982).

Materials and methods:

Groups of 50 male and 50 female mice (CF1/W74, source: [redacted]) received cyfluthrin via the feed for 23 months in concentrations of 0, 50, 200, and 800 ppm, corresponding to 11.6, 45.8, 194.5 mg/kg bw/d in males and 15.3, 63.0, 259.9 mg/kg bw/d in females.
The test compound was a composite sample of 5 different batches, available as a pre-mix concentrate with Wessalon S (from August 1981 onward with 1 % peanut oil) with a cyfluthrin content of approx. 50 %. The purity of individual batches was not given.
Statistical methods: The values of the treated groups were compared with the control values by the Wilcoxon-Mann-Whitney U-test. The mortality rates were compared by Fischers exact test.

Results and discussions:

Findings:
Mortality, clinical signs and body weight: The animals treated with the test substance did not exhibit any differences from the controls in terms of appearance, behaviour and food consumption.
At 200 and 800 ppm mortality was slightly increased in females (equivocal) after 12 months. The dose of 50 ppm had no influence on growth. The dose of 200 ppm produced a slight, transient retardation of growth in males (for example see week 86). At 800 ppm the male and female mice body weights were lower than those of the controls throughout the study.

Table B.6.5-7: Mortality and body weight

Dose [ppm] Sex	0 M/F	50 M/F	200 M/F	800 M/F
Mortality (99 wk) [%]	80/52	78/60	82/74	88/68
Body weight (51 wk) [g] +SD [g] Signi	43.0/34.9 6.1/4.1	40.9/33.9 5.0/3.3	41.9/34.1 4.1/3.4	39.7/33.2 3.2/3.0 */*
Body weight (86 wk) [g] +SD [g] Signi	43.6/36.3 6.0/5.6	40.4/34.6 5.2/3.6	38.8/35.0 4.3/4.4 **/.	38.9/33.4 3.5/4.0 */*

* = p <0.05; ** = p <0.01.

Clinical laboratory tests: The haematological tests, fluoride examination and urinalysis revealed no evidence of dose related toxic effects. The clinical chemical analyses showed increased alkaline phosphatase activities (AP) in males after 6, 12 and 18 months at all tested dose levels.

Table B.6.5-8: Clinical laboratory tests

Dose [ppm] Sex	0 M/F	50 M/F	200 M/F	800 M/F
AP (6 mo)[U/L] +SD [U/L] Signi	59/155 14/45	80/124 18/32 */.	91/120 25/39 **/	144/152 72/56 **/.
AP (12 mo) [U/L] +SD [U/L] Signi	84/163 16/43	115/122 35/40 */*	120/150 42/60	146/117 63/29 **/*
AP (18 mo) [U/L] +SD [U/L] Signi	95/162 16/54	204/193 151/101	153/259 78/135 */*	158/153 59/44 **/

* = p <0.05; ** = p <0.01.

Pathological examinations: Macroscopic-anatomical examinations, organ weight determinations and histopathological findings did not reveal any evidence of a specific organotoxic effect of cyfluthrin at doses up to and including 800 ppm.
No evidence of an oncogenic potential of the substance at any dose level was derived from the type, localisation, incidence and latency of neoplasias found.

Table B.6.5-9: Histopathology, neoplastic lesions

Dose [ppm] Sex	0 M/F	50 M/F	200 M/F	800 M/F
Adrenals [no#] -cort. tumor, non invas.(b) -cort. tumor, invasive (b) -medullary tumor (b)	48/48 0/0 4/1 0/1	43/44 2/1 2/1 1/0	47/47 2/0 2/1 0/0	46/46 1/0 3/0 0/0
Colon [no#] -polyp (b)	33/44 0/0	43/45 0/0	49/48 0/0	43/46 1/0
Connective tissue [no#] -leiomyosarcoma (m)	1/1 0/0	0/1 0/0	1/4 1/2	1/4 0/0
Ileum [no#] -leiomyosarcoma	25/39 0/0	26/34 0/0	27/31 0/1	29/38 0/0
Kidneys [no#] -tub. carcinoma (m)	45/48 1/0	43/45 0/0	49/48 0/0	43/46 0/0

Dose [ppm] Sex	0 M/F	50 M/F	200 M/F	800 M/F
Liver [no#] -adenoma (b) -carcinoma (m)	44/47 0/3 6/2	43/45 2/2 10/1	48/47 3/4 5/1	45/46 4/0 4/3
Lung [no#] -bronchio-alveolar tumor(b) -bronchio-alveolar tumor(m)	46/48 8/14 2/2	44/45 7/3 6/2	49/48 8/6 3/5	48/47 5/12 3/0
Mammary glands [no#] -carcinoma (m)	0/2 0/2	0/2 0/2	0/0 0/0	0/0 0/0
Ovaries [no#] -gran. theca cell tumor (b)	0/48 0/3	0/44 0/1	0/48 0/2	0/46 0/2
Pituitary [no#] -adenoma (b)	40/44 0/4	33/41 0/1	42/43 1/2	39/37 1/1
Reticulohistio. system [no#] -malignant lymphoma (m)	47/48 7/12	45/46 5/11	49/49 9/10	49/47 3/12
Salivary glands -myoepithelioma (b)	42/47 0/0	41/44 0/0	49/47 0/1	43/45 0/0
Skin and adnexa [no#] -carcinoma (m) -leiomyosarcoma (m) -polymorph. sarcoma (m)	1/7 0/0 0/0 0/0	4/9 0/0 0/2 0/0	1/9 0/1 0/1 0/0	10/10 0/0 0/0 1/0
Stomach [no#] -carcinoma (m) -sarcoma (m)	38/47 0/0 0/0	38/44 0/1 0/0	45/44 0/0 1/0	46/46 0/0 1/0
Testes -Leydig's cell tumor (b)	46/0 1/0	42/0 0/0	49/0 0/0	47/0 0/0
Thyroid [no#] -adenoma (b)	42/48 1/1	41/44 0/0	47/47 0/0	41/45 1/2
Uterus [no#] -carcinoma (m) -leiomyoma (b) -leiomyosarcoma (m) -stroma polyp (b) -stroma sarcoma (m)	0/48 0/0 0/2 0/2 0/0 0/1	0/45 0/1 0/0 0/1 0/2 0/0	0/48 0/2 0/0 0/1 0/3 0/0	0/46 0/0 0/0 0/1 0/1 0/0

[no#] Number of organs examined;(b) benign; (m) malignant

In the addendum Suberg and Löser, 1983, TOX9401905 (September 22, 1994) data not reported in the original report were given, i.e. results of clinical observations of individual animals, information about the test substance and about analyses of the mixtures of the test substance (stability, homogeneity, concentration in the mixture), supplementary information on histopathological investigations (brain, spinal cord, parathyroids, cecum and rectum).

Conclusion:

Doses of up to 800 ppm were negative for carcinogenicity. The dose of 50 ppm, equal to 11.60 mg/kg bw/d in males, was classified as NOAEL, because the temporary increase in enzyme activity for alkaline phosphatase at the lowest dose level in the males was different from the parallel findings in treated females, and did not correlate with gravimetric, gross anatomical or histopathological findings. Accordingly there were no indications of liver damage in the doses up to and including 800 ppm in the chronic studies both and also in the short term studies in mice.

Re-evaluation by the RMS (2015):

No evidence of carcinogenicity was observed at all dose levels tested.
800 ppm: Lower body weights of male and female mice throughout the study. Increased alkaline phosphatase activities (AP) in males after 6, 12 and 18 months.
200 ppm: Slight, transient retardation of growth in males. Increased alkaline phosphatase activities (AP) in males after 6, 12 and 18 months.
50 ppm: Increased alkaline phosphatase activities (AP) in males after 6, 12 and 18 months.
The assessment of the study has changed from the initial evaluation in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), when the study was considered acceptable. At present the study cannot be considered acceptable anymore. The main reason is the missing information on the purity of the test substance cyfluthrin (see also deviations).

Studies evaluated in the addendum 1 to the monograph (2002, ASB2014-9599):

Data point:	KCA 5.5
Report:	[redacted], 1997, TOX9850068: Technical grade cyfluthrin: a combined chronic toxicity / oncogenicity testing study in the rat Report-No.: 107769, Study-No.: 94-272-BK, Bayer-File-No. 8384, unpublished [redacted] (Experimental work from 2 December 1994 – December 1996)
Guideline(s):	Yes (OECD Guideline No. 453)
Deviations:	None that were considered to have compromised the validity of the study results (In addition: ophthalmologic examinations were conducted on all acclimatised animals prior to exposure)
GLP:	Yes
Acceptability:	Acceptable

Data point:	KIIA 5.5.2
Report:	[redacted], 2000, TOX2001-1766: Wahle B.S. and Christenson W.R. (2000) Supplemental Submission to Bayer Report No. 107769 Technical Grade Cyfluthrin: A Combined Chronic Toxicity / Oncogenicity Testing Study in Rats. Report-No.: 107769-1, Study-No.: 94-272-BK, Bayer-File-No. 8384, unpublished, [redacted]
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Acceptable

Material and Methods

Test material: Technical grade cyfluthrin, purity: 93.9–95.1 %, batch no.: 4030059/BF9340-71
Test animals: Fischer-344 rats (CDF[F-344]/BR), age: 8 weeks at treatment initiation, Source: [redacted]
Technical grade cyfluthrin was administered to separate 1-year and 2-year sacrifice groups of Fischer 344 rats at nominal dietary concentrations of 0–50–225–450 ppm. The 1-year sacrifice group consisted of 40 animals (20 males and 20 females) in both the control and high-dose groups and 20 animals (10 males and 10 females) in both the low and intermediate dose levels for a total of 120 animals. The 2-year sacrifice group consisted of 100 animals (50 males and 50 females) in all 4 dose groups for a total of 400 animals.
Haematological and clinical-chemistry examinations including urinalyses were performed on the first 20 surviving rats/sex/dose of the 2-year sacrifice group. In all cases, blood was sampled via the orbital sinus following an overnight fast; to the extent possible, urine was collected on the same non-fasted animals the week prior to blood collection.
In addition to the routine guideline requirements, ophthalmologic exams were conducted on all acclimatised animals prior to exposure, and then again on all surviving animals just prior to termination of the 1- and 2-year segments of the study.
At necropsy, the organ weights and organ/body weights were determined for the following tissues: adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen and testicles. All required tissues plus all gross lesions detected at necroscopy from all animals were histopathologically examined.

Results and discussions:

Findings:
Concentration, stability and homogeneity of the test compound in feed
The mean treatment concentrations remained within approx. 5 % of the nominal concentrations. Based on analytical chemistry determinations, cyfluthrin was considered to be homogeneously distributed and stable in the feed.
Cyfluthrin intake:
The mean test substance intake over the 2-year treatment period is summarised in the table below.

Table B.6.5-10: Rat 2-year study: Calculated test substance intake

Nominal dose levels (ppm)	Average daily consumption of cyfluthrin (mg/kg bw/d)	
	Males	**Females**
0	0.0	0.0
50	2.6	3.3
225	11.6	14.4
450	22.8	28.3

Survival:
Survival was unaffected by administration of the test substance as the incidence of mortality was comparable between treated and control animals of each sex. Overall, survival to the end of the 2-year treatment period was in the range of 54–82 %.
Body weight gain and food consumption:
Data for body weight gain and terminal body weight are summarised in the next table.

Table B.6.5-11: Rat 2-yr study: Body weight gain and terminal body weight (24-months group)

Time period	Mean bw gain (g) during the designated study periods at dose (ppm)							
	0	50	225	450	0	50	225	450
	Males				Females			
wk 1 – wk 13	140.5 (100 %)	136.9 (97 %)	123.9 (88 %)	108.8 (77 %)	54.7 (100 %)	53.3 (97 %)	51.2 (94 %)	45.0 (82 %)
wk 1 – wk 26	181.4 (100 %)	175.9 (97 %)	160.4 (88 %)	145.6 (80 %)	73.3 (100 %)	71.8 (98 %)	68.3 (93 %)	58.3 (80 %)
wk 1 – wk 52	224.0 (100 %)	217.8 (97 %)	196.9 (88 %)	173.3 (77 %)	92.0 (100 %)	89.4 (97 %)	86.8 (94 %)	73.9 (80 %)
wk 1 –wk 104[a]	192.1 (100 %)	180.8 (94 %)	171.9 (89 %)	165.0 (86 %)	149.9 (100 %)	137.9 (92 %)	134.7 (90 %)	118.8 (79 %)
Terminal body weight (g)	366.7 (100 %)	354.5 (97 %)	344.4* (94 %)	340.5* (93 %)	274.5 (100 %)	263.8 (96 %)	256.2* (93 %)	236.3* (86 %)

[a] Last body weight determinations for females were performed during treatment week 103.
Statistics: Anova + Dunnett's test: * = $p \leq 0.05$

Body weight gain remained unaffected in both sexes at the low dose level of 50 ppm. At the end of the treatment period, declines of 11 % and 10 % body weight gain were noted in 225-ppm males and females, respectively, while at 450 ppm, body weight gains were reduced by 14 % and 21 % in males and females, respectively. Terminal body weights were statistically significantly decreased at 225 ppm and above in both sexes.
Food consumption and utilisation was not influenced by treatment in both sexes at all doses tested.
Clinical observations and ophthalmoscopy:
With the exception of a statistically significantly increased frequency of alopecia noted in 450-ppm males and females (see table below), neither clinical and/or cage-side observations, nor ophthalmic toxicity attributable to exposure to the test substance were observed.

Table B.6.5-12: Rat 2-yr study: Clinical observations

Group	Incidence of alopecia (skin, forelimb) at dose (ppm)							
	0 (%)	50 (%)	225 (%)	450 (%)	0 (%)	50 (%)	225 (%)	450 (%)
	Males				Females			
1-year group (days 30-367)	0/20 (0)	2/10 (20)	4/10 (40)	13/20 (65)	5/20 (25)	0/10 (0)	5/10 (50)	11/20 (55)
2-year group (days 30-738)	17/50 (34)	16/50 (32)	17/50 (34)	30/50 (60)	30/50 (60)	35/50 (70)	34/50 68)	43/50 (86)

Clinical chemistry, haematology, and urinalysis:
Slightly reduced red blood cell count (RBC), hemoglobin concentration and haematocrit were noted at the 3- and 6-months investigation in males and at the 6-months investigation in females of the 450 ppm group. In males also the lower dose groups revealed minimally lower values during the first 6 months of treatment. Later investigations revealed no differences between the dose groups anymore. Reticulocytes counts were distributed uniformly between all dose groups and at all investigations. Therefore, no toxicological significance was attributed to these findings.
Clinical chemistry findings included a slight decline in serum triglyceride concentration in 225- and 450-ppm males at all investigations. Statistical significance was mainly reached for the high dose group (450 ppm). To a lesser extent serum cholesterol concentration was also reduced (3-, 12-, 24-months investigation). In females a tendency to slightly lower triglyceride values was also noted at 6-, 18- and 24-months investigation. Cholesterol values are comparable between the groups.
No evidence of cyfluthrin-induced toxicity was observed in any other in-life parameters including

urinalysis. No treatment-related gross lesions were observed at the 12-months and 24-months necropsy.

Organ weight changes:

Statistically significant changes in absolute organ weights and organ/body weight ratios are summarised in the table below. Decreased absolute weights were accompanied by increases in the respective relative organ weights, indicating that the organ weight changes observed in this study were secondary to cyfluthrin-induced decreases in body weight. This conclusion is supported by the lack of corresponding treatment-related histopathological tissue changes.

Table B.6.5-13: Rat 2-year study: Organ weight changes (24-months group)

Parameter	Dose (ppm)			
	0	50	225	450
MALES				
Adrenals abs. wt (g)	0.088 (100 %)	0.085 (97 %)	0.072$ (82 %)	0.070$ (80 %)
rel. wt (%)	0.013 (100 %)	0.013 (100 %)	0.014 (108 %)	0.014* (108 %)
Kidneys abs. wt (g)	3.587 (100 %)	3.586 (100 %)	3.341* (93 %)	3.287* (92 %)
rel. wt (%)	0.799 (100 %)	0.830 (104 %)	0.846* (106 %)	0.873* (109 %)
Liver abs. wt (g)	18.29 (100 %)	17.08 (93 %)	15.95* (87 %)	14.73* (81 %)
rel. wt (%)	3.778 (100 %)	3.881 (103 %)	3.980 (105 %)	4.096* (108 %)
FEMALES				
Liver abs. wt (g)	11.47 (100 %)	11.32 (99 %)	11.08 (97 %)	10.05$ (88 %)
rel. wt (%)	4.097 (100 %)	4.119 (101 %)	4.067 (99 %)	4.217 (103 %)

Statistics: Anova + Dunnett's test: * = $p \leq 0.05$;
Kruskal-Wallis Anova + Mann-Whitney u-test: $ = $p \leq 0.05$.

Histopathology:

At 12-month investigation no treatment-related histopathological changes were observed. There were no neoplastic or non-neoplastic microscopic alterations in the 24-month male and female rats that were considered to be compound-related. Only one neoplasm was marginally increased over the concurrent controls consisting of mammary gland adenocarcinomas in the 24-month 450 ppm female rats (see next table).

Table B.6.5-14: Rat 2-year study: Findings in the female mammary gland

MAMMARY GLAND	Incidence of mammary gland lesions (animals with lesion / animals examined)					
	Dose level (ppm)				Historical control dataa	
	0	50	225	450	92-272-SC	91-272-LJ
Hyperplasia	0/50	1/50	0/50	2/50	0/50	0/50
Adenomas	0/50	0/50	0/50	0/50	0/50	1/50
Adenocarcinoma	1/50	0/50	0/50	4/50	0/50	1/50
Fibroadenoma	9/50	15/50	9/50	4/50	no data	no data
Total mammary gland tumours	10/50	15/50	9/50	8/50	no data	no data

[a] Historical control data was available from two 2-year studies conducted at the testing facility using the Fischer-344 rat (Study-No. 92-272-SC and 91-272-LJ)

Despite being out of range of in-house historical control data, the increased incidence of mammary gland adenocarcinomas was considered to be incidental for the following reasons:

There was no suggestion of compound-induced carcinogenicity due to cell proliferation based on the

incidence of mammary gland hyperplasias, fibroadenomas, and a lack of mammary gland adenomas; No dose-dependent increase incidence of all mammary gland tumours combined was found. Additionally a complete battery of mutagenicity studies performed on the compound indicated that cyfluthrin was non-genotoxic. The time to tumour development between control and treated animals appeared to be comparable, as no proliferative lesions of any kind were seen in the mammary glands of the 12-month group in this study and all treated and control 24-month females that contained mammary gland adenocarcinomas were sacrificed at study termination. Finally, there was no evidence of compound-induced carcinogenicity based on a previous two-year feeding study in the Wistar rat with technical grade cyfluthrin at doses identical to those used in this study.

Wahle B.S. and Christenson W.R. (2000, TOX2001-1766) Supplemental Submission to Bayer Report No. 107769 (Report-No.: 107769-1): The report 107769 (Technical Grade Cyfluthrin: A Combined Chronic Toxicity / Oncogenicity Testing Study in Rats) was classified as unacceptable by the California authorities. Several issues were discussed between the California authorities and the notifier. The supplemental submission mainly deals with the rationale for dose selection which was not accepted by the California authorities since there was no treatment-related effect in the study except body weight reduction. The notifier clearly feels that an MTD was achieved in this study, based principally upon the adverse decline in body weight.

Conclusion

Based on the lack of adverse compound-related effect in body weight gain at a dose of 50 ppm in males and females, a systemic chronic toxicity NOEL of 2.6 and 3.3 mg cyfluthrin/kg bw/d was established for male and female rats, respectively. No evidence for compound-induced neoplasia was found in this study.

Re-evaluation by the RMS (2015):

Body weight reduction at 225- and 450-ppm males and females. NOAEL: 50 ppm (2.6/3.3 mg/kg bw/d).
The study is considered acceptable under the conditions of the study and based on the information given in the report and in the supplemental submission (Report No. 107769-1 of Wahle B.S. and Christenson W.R., 2000, TOX2001-1766). Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

Data point:	KCA 5.5.3
Report:	[redacted], 1998, TOX2001-1770 Technical grade cyfluthrin: An oncogenicity study in the mouse, [redacted]., Study No. 95-271-DR, Report No. 108041, unpublished, (Experimental work from 15 November 1995 – 21 May 1997) [redacted] (2000) TOX2001-1770, Supplemental submission to Bayer report No. 108041-1[1], Technical grade cyfluthrin: An oncogenicity study in the mouse, [redacted]., Study No. 95-271-DR, Report No. 108041-1, unpublished
Guideline(s):	Yes (OECD Guideline No. 451)
Deviations:	None that were considered to have compromised the validity of the study results

[1] During the evaluation of Report No. 108041 an electronic request was made (8/16/00) for a separate summary table including only neoplastic lesions (in addition to the micropathology table listing both non-neoplastic and neoplastic lesions, already included in the report). Subsequently, an addendum to the original report was prepared with an extra table containing only neoplastic lesions.

GLP: Yes

Acceptability: Acceptable

Material and Methods:

Test material: Cyfluthrin, technical grade, purity: 93.9–95.1 %, batch no.: 4030059/BF9340-71
Test animals: CD-1 mice, age and bw (Day 0): approx. 8 weeks; males: 28.7 g, females: 24.3 g
Source: [redacted]
Cyfluthrin was administered in the diet to 50 CD-1 mice per dose and sex for approx. 18 months. Nominal doses: 0–200–750–1400/1600 ppm (male/female), equivalent to 0–31.9–115–233 mg/kg bw/d for males and 0–38.4–141–310 mg/kg bw/d for females). All test diets were available for *ad libitum* consumption at all times; the homogeneity and stability of cyfluthrin as a dietary mixture was confirmed. Body weight and food consumption determinations were conducted weekly for approx. 17 months and once during the last month of the study; detailed clinical examinations of each animal were conducted weekly throughout the study. Standard haematological and differential leukocyte analyses were performed on blood from non-fasted animals at approx. 12 and 18 months into the study. All animals placed on study were subject to a post-mortem examination, which included (1) documenting and saving all gross lesions, (2) weighing designated organs (adrenal glands, brain, heart, kidneys, liver, lungs, ovaries, spleen and testes), and (3) collecting representative tissue specimens for histopathological evaluation.

Results and discussions:

Findings:
Body weight gain and food consumption:
Decreased body weight gains over the 18-month treatment period (see next table) were observed in all female treatment groups and in high-dose-group males. Food consumption remained unaffected in both sexes at all doses tested. At sacrifice, female terminal body weights were statistically significantly decreased compared to controls at all dose levels tested, while male terminal body weight was statistically significantly decreased only at 1400 ppm.

Table B.6.5-15: Mouse 18-month carcinogenicity study: body weight gains

Parameter	Male dose groups (ppm)				Female dose groups (ppm)			
	0	200	750	1400	0	200	750	1600
Bw gain (g) 0–18 mo	11.3 (100 %)	10.9 (95 %)	10.4 (95 %)	8.5 (77 %)	13.2 (100 %)	11.8 (91 %)	10.4 (80 %)	6.1 (46 %)
Terminal bw (g)	39.3 (100 %)	38.7 (98 %)	37.6 (96 %)	35.8* (91 %)	36.4 (100 %)	34.0* (93 %)	33.1* (91 %)	29.8* (82 %)

* Statistically significant (Anova + Dunnett's Test): $p \leq 0.05$

Clinical observations attributable to exposure included alopecia and rough coat in the 1400/1600 ppm males and females, and hunched back, lesion redness, and lesion scab observed in the 1600 ppm females. The redness and scabs were generally associated with the ear pinnae of one or both ears.

Table B.6.5-16: Mouse 18-month carcinogenicity study: clinical observations

Parameter	Male dose groups (ppm)				Female dose groups (ppm)			
	0	200	750	1400	0	200	750	1600
Alopecia	5/50	8/50	6/50	11/50	14/50	17/50	18/50	12/50
Rough coat	9/50	18/50	16/50	30/50	9/50	5/50	7/50	20/50
Hunched back	0/50	1/50	1/50	0/50	0/50	3/50	0/50	5/50
Lesion, redness	4/50	1/50	2/50	4/50	0/50	2/50	1/50	5/50
Lesion, scab	16/50	16/50	22/50	20/50	2/50	7/50	7/50	22/50

No evidence of a cyfluthrin-induced toxicity was observed in any other in-life parameter including survival and haematology.
Gross pathological observations attributable to exposure included rough coat in 1400/1600 ppm males and females, crusty zones of the skin of the ear in 750 ppm males and the 1400/1600 ppm males and females, and wet/stained ventrum in 1400 ppm males. Numerous declines in absolute organ weight were observed especially in female treatment groups (see next two tables).

Table B.6.5-17: Mouse 18-month carcinogenicity study: Male terminal body weight and organ weights

Parameter		Dose (ppm)							
		0		200		750		1400	
MALES									
Terminal bw [g]		39.3	(100 %)	38.7	(98 %)	37.6	(96 %)	35.8*	(91 %)
Brain	abs. wt (g)	0.517	(100 %)	0.511	(99 %)	0.514	(99 %)	0.512	(99 %)
	rel. wt (%)	1.331	(100 %)	1.333	(100 %)	1.376	(103 %)	1.442*	(108 %)
Heart	abs. wt (g)	0.232	(100 %)	0.235	(101 %)	0.241	(104 %)	0.235	(101 %)
	rel. wt (%)	0.596	(100 %)	0.610	(102 %)	0.643	(108 %)	0.656	(110 %)
Kidney	abs. wt (g)	0.924	(100 %)	0.875	(95 %)	0.907	(98 %)	0.903	(98 %)
	rel. wt (%)	2.353	(100 %)	2.272	(97 %)	2.413	(103 %)	2.527*	(107 %)
Liver	abs. wt (g)	2.371	(100 %)	2.294	(97 %)	2.253	(95 %)	2.245	(95 %)
	rel. wt (%)	6.059	(100 %)	5.940	(98 %)	5.996	(99 %)	6.318$	(104 %)
Spleen	abs. wt (g)	0.145	(100 %)	0.124	(86 %)	0.113	(78 %)	0.106$	(73 %)
	rel. wt (%)	0.372	(100 %)	0.322	(87 %)	0.303	(81 %)	0.296	(80 %)
Testes	abs. wt (g)	0.226	(100 %)	0.223	(99 %)	0.239	(106 %)	0.226	(100 %)
	rel. wt (%)	0.576	(100 %)	0.582	(101 %)	0.637*	(111 %)	0.637*	(111 %)

* Statistically significant (Anova + Dunnett's Test): $p \leq 0.05$
$ Statistically significant (Kruskal-Wallis + Mann-Whitney u-Test): $p \leq 0.05$

Table B.6.5-18: Mouse 18-month carcinogenicity study: Female terminal body weight and organ weights

Parameter		Dose (ppm)							
		0		200		750		1400 / 1600	
FEMALES									
Terminal bw (g)		36.4	(100 %)	34.0*	(93 %)	33.1*	(91 %)	29.8*	(82 %)
Brain	abs. wt (g)	0.529	(100 %)	0.527	(100 %)	0.530	(100 %)	0.512*	(97 %)
	rel. wt (%)	1.464	(100 %)	1.563*	(107 %)	1.612*	(110 %)	1.742*	(119 %)
Heart	abs. wt (g)	0.201	(100 %)	0.192	(96 %)	0.197	(98 %)	0.171*	(85 %)
	rel. wt (%)	0.555	(100 %)	0.567	(102 %)	0.594	(107 %)	0.582	(105 %)
Kidney	abs. wt (g)	0.669	(100 %)	0.606	(91 %)	0.630	(94 %)	0.579	(87 %)
	rel. wt (%)	0.810	(100 %)	0.810	(100 %)	0.877$	(108 %)	0.877$	(108 %)
Liver	abs. wt (g)	2.171	(100 %)	1.971*	(91 %)	2.112	(97 %)	1.938*	(89 %)
	rel. wt (%)	5.946	(100 %)	5.796	(97 %)	6.350	(107 %)	6.520*	(110 %)
Lung	abs. wt (%)	0.295	(100 %)	0.275	(93 %)	0.291	(99 %)	0.260	(88 %)
	rel. wt (%)	0.810	(100 %)	0.810	(100 %)	0.877$	(108 %)	0.877$	(108 %)
Ovary	abs. wt (g)	0.163	(100 %)	0.214	(131 %)	0.182	(112 %)	0.122$	(75 %)
	rel. wt (%)	0.442	(100 %)	0.650	(147%)	0.562	(127 %)	0.372$	(84 %)
Spleen	abs. wt (g)	0.205	(100 %)	0.152$	(74 %)	0.141	(69 %)	0.128$	(62 %)
	rel. wt (%)	0.554	(100 %)	0.448	(81 %)	0.423	(76 %)	0.429	(77 %)

* Statistically significant (Anova + Dunnett's Test): $p \leq 0.05$
$ Statistically significant (Kruskal-Wallis + Mann-Whitney u-Test): $p \leq 0.05$

Evaluation of organ/body weight ratios suggest that organ weight changes observed in this study were likely secondary to cyfluthrin-induced decreases in body weight gain. This conclusion is supported by the lack of microscopic evidence of a direct toxicological insult by cyfluthrin on any tissue examined in this study.
Microscopic lesions associated with exposure to the test substance observed in this study occurred in a gross lesion involving the skin of the ear and included acanthosis, chronic active inflammation, inflammation–all types, ulcer, and debris, which corresponded to the increased incidence of "crusty zones" found at the tip of the ears upon gross necropsy examination. The incidences were generally elevated in 750-ppm males and 1400/1600-ppm males and females. In general, the affected ears at the time of necropsy were ulcerated (parts of pinnae missing) and red with crust and debris. The "skin ear" (tip of ear) lesions appear to have resulted from cyfluthrin-induced paraesthesia.
The body weight profile which emerged through approx. 18 months of continuous and repeated dietary exposure to the test substance suggests that at the highest dose tested, the MTD for cyfluthrin in the male mouse was established (1400 ppm), while in the female mouse, the MTD was clearly exceeded (1600 ppm). No evidence of a compound-induced neoplastic response was observed in any tissue examined.

Conclusion

Under the conditions of the this study, cyfluthrin showed no evidence of a carcinogenic potential in mice after 18-month continuous dietary exposure of up to 1400 ppm in males and 1600 ppm in females, the highest dose tested. A NOAEL for systemic toxicity could not be derived because female body weights were slightly albeit statistically significantly decreased already at 200 ppm, the lowest dose level tested. In males, a NOAEL for systemic toxicity of 200 ppm (31.9 mg/kg bw/d) was based on increased incidences of crusty ear lesions at and above 750 ppm (115 mg/kg bw/d).
NOEL (Mouse 18-month carcinogenicity): 1400 ppm (233 mg/kg bw/d).

Re-evaluation by the RMS (2015):

No evidence of carcinogenicity was observed at all dose levels tested.
200 ppm (38.4 mg/kg bw/d): Decreased body weight gains in females.
750 ppm (115/141 mg/kg bw/d): Decreased body weight gains in females; Gross pathological observations: crusty zones of the skin of the ear in males; Histopathology: acanthosis, chronic active inflammation, inflammation–all types, ulcer, and debris (corresponding to "crusty zones" found at the tip of the ears upon gross necropsy examination) in males.
1400 ppm (233 mg/kg bw/d)/1600 ppm (310 mg/kg bw/d): Decreased body weight gains in males and females; clinical observations: alopecia and rough coat in males and females; hunched back, lesion redness, and lesion scab in females; Gross pathological observations: rough coat and crusty zones of the skin of the ear in males and females, wet/stained ventrum in males; Histopathology: acanthosis, chronic active inflammation, inflammation–all types, ulcer, and debris (corresponding to "crusty zones" found at the tip of the ears upon gross necropsy examination) in males and females.
Based on the reduced body weight in female animals from 200 ppm onwards, no NOAEL can be derived for females from this study. The NOAEL in males was 32 mg/kg/day.
The NOAEL for carcinogenicity is 1400/1600 ppm (233/310 mg/kg bw/d).
A NOAEL for systemic effects could not be derived based on decreased body weight gains in females at all dose levels.
The study is considered acceptable under the conditions of the study and based on the information given in the report and in the supplemental submission Wahle and Christenson, 1998 and 2000, TOX2001-1770 (Report No. 107769-1). Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

B.6.6 Reproductive toxicity

No new data on reproductive toxicity have been generated since the Annex-I inclusion of cyfluthrin/beta-cyfluthrin and the publication of the addendum 1 (2002, ASB2014-9599). All studies here have been previously submitted. For the renewal process they were again evaluated.
After Annex I inclusion a developmental neurotoxicity screening study with beta-cyfluthrin in rats has been conducted (Sheets, 2003, ASB2007-2856, reported under B.5.7).
A literature research for the Renewal Assessment Report (RAR) including publications from the last 10 years was performed by the RMS. The publications were considered as supplemental information or not acceptable. The results had no influence on the derivation of threshold values or on classification and labelling of beta-cyfluthrin.

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

B.6.6.1 Generational studies

Data point:	KCA 5.6.1
Report:	[redacted], 1983, TOX9401906: FCR 1272 - Multigeneration study on rats. Report no.: 11870 (June 08, 1983, report), 11870A (December 07, 1987, addendum); [redacted]
Guideline(s):	The performance of the test was based on recommendations published by the FDA (1968, 1980).
Deviations:	The purity of individual batches of the test compound was not given; 20 pregnant females were not present in every test group; water consumption is not reported; the presence of sperm or vaginal plug was not checked during mating; sperm parameters and estrus cycle length were not taken/ reported; mating was performed with one male and two females in on cage; physical development of offspring and sexual maturation was not reported (i.e. ear/eye opening); functional investigation (motor activity, sensory function) of offspring was not performed; parental animals (P and F1) were, with the exception of intercurrently died animals, not examined macroscopically and histologically; the following organ weights were not taken: uterus, epididymides, prostate, seminal vesicles, brain, spleen, pituitary, adrenal and thyroid gland,
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Not acceptable

(Dates of exp. work: November 1980 - November 1982).

Materials and methods:

Groups of 10 male and 20 female Wistar rats (Bor:WISW (SPFcpb), [redacted]; body weight 85-90 g) received cyfluthrin (purity not reported) via the feed in concentrations of 0, 50, 150, and 450 ppm corresponding to 0, 3.80-3.95, 11.37-13.58, 34.74-39.58 mg/kg bw for males and 0, 5.14-5.53, 14.01-15.96, 46.86-50.16 mg/kg bw for females throughout the entire experimental period. The test compound was a composite sample of 5 different batches, available as a pre-mix concentrate with Wessalon S with a cyfluthrin content of approx. 50 %. The purity of individual batches was not given.
Statistical methods: U-test of Mann, Whitney and Wilcoxon, Fishers exact test.

Results and discussions:

Doses of up to and including 450 ppm had no effect on the mortality, appearance and behaviour of the F0, F1b, and F2b parents. For females, dose levels of 50 ppm did not result in relevant treatment related influences on body weight gain. But the F1b males of the 50 ppm dose group gained weight more slowly than males of the control group. These weight differences were significant at weeks 22, 23, 30 and after week 35 (for example see Table B.6.6-1). At 150 ppm and 450 ppm the decreased body weight gains of parental rats were evident. No relevant differences in food consumption can be found in rats up to a dose level of 450 ppm in F0-generation. At males F1b-generation food consumption was markedly reduced at 450 ppm (19 %) and slightly at 150 ppm (14 %).

Table B.6.6-1: Body weight (parental data)

Dose [ppm] Sex	**0 M/F**	**50 M/F**	**150 M/F**	**450 M/F**
Means(F0, 31 wk) [g] +SD [g] Signi	371/263 19/44	392/264 24/32	360/241 15/36 */-	343/224 22/25 **/**
Means(F1b, 30 wk) [g] +SD [g] Signi	411/212 47/22	370/220 40/21 */-	323/210 47/19 **/-	329/195 17/14 **/**
Means(F2b, 29 wk) [g] +SD [g] Signi	389/223 27/16	404/219 30/15	372/211 23/17 **/*	338/205 37/13 **/**

* = p <0.05, ** p <0.01.

The fertility index for 450 ppm females of the 2nd mating of the F1b generation was lower when compared to lower dose groups and the first mating (1st mating; dose groups 0-50-150-450 ppm: 90-100-90-85 %; 2nd mating: 85-90-85-65 %).
Doses of up to and including 450 ppm did not induce malformations in the pups, and did not give rise to any anomalies in the male/female ratio.
The treatment with FCR 1272 at 150 and 450 ppm resulted in reductions in viability and lactation index (Table B.6.6-2), occasionally fewer pups per litter and decreased body weight gains in the pups. In F1b, the total number of pups was decreased at 150 and 450 ppm and the number of dead pups was increased at 450 ppm. One pup in the 150 ppm group and some pups in the 450 ppm group exhibited convulsions.

Table B.6.6-2: Viability index [VI], lactation index [LI]:

Dose [ppm]	**0**	**50**	**150**	**450**
F1a-VI [%] F1a-LI [%]	100 99.5	99.5 97.7	93.9** 97.2	96.7* 87.1**
F1b-VI [%] F1b-LI [%]	91.2 96.0	98.0** 95.5	97.6* 91.4	91.4 83.5**
F2a-VI [%] F2a-LI [%]	98.6 95.1	96.2 91.9	94.1 91.8	91.9* 80.2**
F2b-VI [%] F2b-LI [%]	88.0 93.1	93.7 92.6	83.2 75.8**	88.0 72.4**
F3a-VI [%] F3a-LI [%]	96.1 94.3	94.1 94.3	77.0** 90.4	77.8** 92.3
F3b-VI [%] F3b-LI [%]	99.0 97.7	92.3** 94.9	89.0** 98.3	77.4** 91.5*

* = p <0.05; ** = p <0.01.

Table B.6.6-3: Total and dead number of pups

Dose [ppm]	0	50	150	450
Total number F1a Dead number of F1a pups	206 0	203 3	215 2	210 1
Total number F1b Dead number of F1b pups	215 0	202 0	167 1	169 6

Table B.6.6-4: Mean birth weight [MBW]

Dose [ppm]	0	50	150	450
F1a-MBW [g]	5.9	5.9	5.5	5.5*
F2a-MBW [g]	6.1	5.6*	5.9	5.5*
F3a-MBW [g]	6.0	5.8	5.8	5.5

* = p <0.05; ** = p <0.01.

The treatment with 450 ppm resulted in a negative effect on the birth weight of the F1a and F2a generations (Table B.6.6-3).
The necropsy of the parents (spontaneous deaths and the sacrificed F2b-animals) and the necropsies and histopathological examination of the organs of F3b offspring revealed no effects at doses up to and including 450 ppm.
The determination of organ weights, which was performed for the F2b generation only, revealed a dose-dependent and, in some cases, significant reduction of the absolute liver and kidney weights.

Table B.6.6-5: Absolute organ weights (ABW) (F2b)

Dose [ppm]		0	50	150	450
Liver	ABW [mg] m ABW [mg] f	13215 8795	13477 8621	12007 7878**	11408* 8026**
Kidney	ABW [mg] m ABW [mg] f	2453 1614	2608 1632	2414 1501**	2293 1482**
Testes Ovaries	ABW [mg] ABW [mg]	3589 146	3602 139	3242 134	3336 134

* = p <0.05; ** = p <0.01.

Conclusion:

A NOAEL of 50 ppm for parental and reproduction toxicity, corresponding to 3.74 mg/kg bw/d in males and to 5.14 mg/kg bw/d in females, was classified for reason of a slight growth retardation in one parent generation and a slightly reduced viability index in the F3b generation. At higher doses a retardation of growth of the parents and pups and a reduced viability of pups were evident. The NOAEL in male rats (3.74 mg/kg bw/d) served as basis for calculation of the proposed AOEL, oral for cyfluthrin.

Re-evaluation by the RMS (2015):

450 ppm (34.74-39.58 mg/kg bw/d for males/46.86-50.16 mg/kg bw/d for females):
Decreased body weight gains of parental rats; lower fertility index; reductions in viability and lactation index; fewer pups per litter and decreased body weight gains in the pups; decreased total number of pups; increased number of dead pups; significant reduction of absolute liver and kidney weights.
150 ppm (11.37-13.58 mg/kg bw/d for males/14.01-15.96 mg/kg bw/d for females):
Decreased body weight gains of parental rats; reductions in viability and lactation index; fewer pups per litter and decreased body weight gains in the pups; decreased total number of pups; significant reduction of absolute liver and kidney weights.

The NOAEL for parental and reproduction toxicity was 50 ppm corresponding to 3.74 mg/kg bw/d in males and to 5.14 mg/kg bw/d in females.
The study is now considered to be not acceptable since no information on the purity of the test substance is given and due to a variety of deviations from the test guideline. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered to be acceptable.
In the addendum to the report no. 11870 (Loeser and Eiben, October 9, 1987, TOX9401906; GLP: yes), the revision of the food consumption data and the approx. duration of the individual phases of the study are reported.

Studies evaluated in the addendum 1 to the monograph (2002, ASB2014-9599):

Data point:	KCA 5.6.1
Report:	[redacted], 1996, TOX2001-1771: A Two-Generation Reproduction Study in Rats Using Technical Grade Cyfluthrin Administered Via the Diet [redacted] Study-No. 93-672-UZ, Bayer File-No.: 7910, unpublished (Experimental work from 8 September 1993 – 15 June 1994)
Guideline(s):	OECD Guideline 416 (adopted January 2001)
Deviations:	Sperm parameters were not evaluated.
GLP:	Yes
Acceptability:	Acceptable

(Experimental work from 8 September 1993 – 15 June 1994)

Materials and methods:

Test material: Technical grade cyfluthrin, purity: 94.6–96.2 %; batch no. 2030025
Test animals: Male and female Sprague-Dawley rats, age at study initiation: 7 weeks; Source: [redacted]
Technical grade cyfluthrin was administered via the diet to Sprague-Dawley rats (30 rats/sex/group) for two generations (one mating per generation) to test for potential reproductive and neonatal effects. The test compound was administered at nominal dose levels of 0–50–125–400 ppm, corresponding to internal dose calculation for males premating / females premating/ gestation/ lactation:
50 ppm: 3.3 mg/ kg bw/d
125 ppm: 8.3 mg/ kg bw/d
400 ppm: 26.7 mg/ kg bw/d.
The F_0 and F_1 adults received cyfluthrin in the diet throughout the entire study, beginning at seven weeks of age for the F_0 adults and at weaning for the F_1 adults. Prior to breeding, the animals received treated feed at least for a ten-week period. During the study, adult animals were evaluated for the effect of the test compound on body weight, food consumption, clinical signs, oestrus cycling, mating, fertility, gestation length, and litter size. The offspring were evaluated for compound-related effects on sex ratio, pup viability, body weight gain, and clinical signs. Gross necropsy evaluations were performed on all adults and pups. Histopathological evaluation of reproductive organs, the pituitary, and gross lesions was performed on all F_0 and F_1 adults. Additionally due to clinical signs of neurotoxicity, the brain, spinal cord, and one sciatic nerve were collected from all F1 adults and placed in buffered 10 percent formalin in the event that further microscopic examination was deemed necessary.

Results and discussions:

Results parental animals:
Test substance intake:
The intake of cyfluthrin, calculated using the analytical concentration of cyfluthrin in the feed and based on body weight and food consumption data, is presented in Table B.6.6-6 below. For risk assessment purposes, a time-weighted conversion factor of 15 was used for calculation of the test substance intake based on the test substance feed concentration, as proposed by the WHO (2000, ASB2013-4646).

Table B.6.6-6: Rat 2-generation study: Test substance intake

Level	Mean doses in mg/kg bw/d				
	Males	Females			Default calculation*
	Premating	Premating	Gestation	Lactation	Males & Females
50 ppm	3	4	4	7	3.3
125 ppm	9	10	10	19	8.3
400 ppm	29	33	33	59	26.7

* based on default conversion factor of 15 proposed by JMPR (WHO) to be used for rat multi-generation studies

Clinical observations:
There were no compound-related clinical signs for adult males. However, for F_0 and F_1 females there was a compound-related splaying of the hind limbs at 400 ppm which occurred during the lactation phase (see next table and discussion below).

Table B.6.6-7: Rat 2-generation study: Incidence of splayed hind limbs in females during lactation

Generation	Incidence of splayed hind limbs in dose group females during lactation			
	0 ppm	50 ppm	125 ppm	400 ppm
F0 females	(0/30)	(0/27)	(0/26)	(15/29)**
F1 females	(0/25)	(0/27)	(0/27)	(9/25)**

Statistically significant (Fisher's Exact Test): * = $p \leq 0.05$; ** = $p \leq 0.01$

Survival:
There were no compound-related mortalities.
Body weight and food consumption:
There was no compound-related effect on body weight for F_0 and F_1 females or F_0 males during the premating period. In mid- and high-dose group F_1 males, however, terminal body weights were statistically decreased by 6 % and 8 %, respectively, while females were affected only after exposure to the high dose of 400 ppm: F_0 females during the gestation phase (-13 % bw gain) and both F_0 and F_1 females during the lactation phase (bw gains decreased by 30 % and 46 % for F_0 and F_1 females, respectively).
There was no compound-related effect on food consumption for males or females (premating and gestation phases). During the lactation period, however, compound-related decreases in food consumption were observed at 125 ppm in F_1 females, and at 400 ppm in both the F_0 and F_1 females.

Table B.6.6-8: Rat 2-generation study: Body weight gains of F_0 and F_1 adults

Dose Level	Body weight gains (g)							
	F0 generation adults				F1 generation adults			
	Males	Females			Males	Females		
	Premating (wk 1–14)	Premating (wk 1–14)	Gestation (day 0–20)	Lactation (day 0–21)	Premating (wk 1–11)	Premating (wk 1–11)	Gestation (day 0–20)	Lactation (day 0–21)
0 ppm	188 (100 %)	75.6 (100 %)	121.8 (100 %)	25.8 (100 %)	196 (100 %)	78.3 (100 %)	112.9 (100 %)	40.9 (100 %)
50 ppm	184 (98 %)	78.4 (104 %)	122.0 (100 %)	23.7 (92 %)	203 (104 %)	82.9 (106 %)	120.8 (107 %)	31.6 (77 %)
125 ppm	173 (92 %)	72.5 (96 %)	108.1 (89 %)	25.8 (100 %)	191 (97 %)	84.4 (108 %)	107.7 (95 %)	29.2 (71 %)
400 ppm	169 (90 %)	64.5 (85 %)	106.3** (87 %)	18.1a (70 %)	181 (92 %)	74.5 (95 %)	100.2 (89 %)	21.1a (54 %)

** = $p \leq 0.01$ (statistically significant according to Dunnett's test)

a body weight of F_0 and F_1 high-dose females significantly reduced compared to control levels on lactation days 4, 7, 14 and 21 (** = $p \leq 0.01$, Dunnett's test)

Reproductive parameters:
There were no compound-related effects on adult reproductive parameters (oestrus cycle staging; insemination length; mating, fertility and gestation indices; gestation length; number of implantation sites and birth index.
Terminal body weights and organ weight changes:
Statistically significantly decreased terminal body weights were observed in F1 males at 125 ppm and 400 ppm and in F1 females at 400 ppm. There were no compound-related absolute or relative organ weight changes in the F_0 and F_1 adults.

Table B.6.6-9: Rat 2-generation study: Terminal body weights of F_0 and F_1 adults

Generation	Terminal body weights (g, mean ± SD)			
	0 ppm	50 ppm	125 ppm	400 ppm
F0 males	411.1 ± 49.5 (100 %)	405.1 ± 42.7 (99 %)	391.5 ± 52.2 (95 %)	392.6 ± 37.4 (95 %)
F0 females	288.9 ± 19.1 (100 %)	286.1 ± 22.6 (99 %)	285.0 ± 22.0 (99 %)	276.9 ± 21.9 (96 %)
F1 males	422.6 ± 29.0 (100 %)	431.4 ± 43.5 (102 %)	396.2 ± 46.1* (94 %)	389.7 ± 46.3* (92 %)
F1 females	289.0 ± 27.4 (100 %)	289.6 ± 26.6 (100 %)	278.5 ± 30.2 (96 %)	266.0 ± 26.7* (92 %)

Statistics: Anova + Dunnett's Test (two-sided); * = $p \leq 0.05$

Gross and histopathological lesions:
No compound-related effects were observed.
Results offspring:
Clinical observations:
Compound-related coarse tremors were observed in the F_1 and F_2 pups at and above 125 ppm (see next table). The tremors were observed as early as lactation day 5 and had ceased by lactation day 18.

Table B.6.6-10: Rat 2-generation study: Litter incidence of coarse tremors

Generation	Litter incidence of coarse tremors in pups observed during lactation			
	0 ppm	50 ppm	125 ppm	400 ppm
F1 pups	(0/30)	(0/27)	(4/25)	(15/28)*
F2 pups	(0/25)	(0/26)	(19/26)*	(9/25)*

Statistics: Chi-square test & Fisher's Exact test (Bonferroni adjustment of the p value)

Pup gender:
There was no compound-related effect on pup gender.
Litter size; live birth, viability and lactation indices:
No compound-related effects.
Birth weight and pup body weight development during lactation:
Cyfluthrin administration to F_0 and F_1 parents had no effect on birth weight of their offspring. Statistically decreased pup weights observed in F_2 pups at 50 and 400 ppm were not considered treatment-related in the absence of a relation to dose, because a corresponding decrease was not observed in F_1 pups and because the values were within the historical control range (see next table).

Table B.6.6-11: Rat 2-generation study: Pup body weight development

Lactation day	Mean body weight of viable pups (g)								
	F1 pups (males + females combined)				F2 pups (males + females combined)				H.C.c
	0 ppm	50 ppm	125 ppm	400 ppm	0 ppm	50 ppm	125 ppm	400 ppm	(range)
1	6.6 (100 %)	6.6 (100 %)	6.4 (97)	6.6 (100 %)	6.7 (100 %)	6.4* (97 %)	6.4 (97 %)	6.3** (95 %)	6.8 (6.1–7.2)
4a	10.1 (100 %)	10.2 (102 %)	9.7 (97 %)	9.2* (92 %)	10.3 (100 %)	9.3* (91 %)	9.5 (92 %)	8.2** (80 %)	10.2 (9.2–11.3)
4b	10.0 (100 %)	10.3 (103 %)	9.7 (97 %)	9.2* (92 %)	10.3 (100 %)	9.3* (91 %)	9.5 (92 %)	8.2** (80 %)	
7	16.2 (100 %)	16.4 (101 %)	15.0* (93 %)	13.7** (85 %)	16.1 (100 %)	14.7* (91 %)	14.4** (89 %)	12.0** (75 %)	16.3 (14.8–18.7)
14	31.4 (100 %)	31.5 (100 %)	29.5* (94 %)	25.2** (80 %)	30.3 (100 %)	28.8 (95 %)	25.8** (85 %)	23.0** (76 %)	32.0 (29.6–35.8)
21	49.0 (100 %)	50.1 (102 %)	46.1 (94 %)	39.4** (80 %)	45.4 (100 %)	42.8 (94 %)	39.0** (86 %)	33.6** (74 %)	50.4 (46.6–56.9)

a before culling; b post culling;
c Historical control data for F_2 pup body weight compiled from 14 studies with Sprague-Dawley rats unequivocally originating from SASCO Inc.; studies conducted between 1988–1995 by Bayer Corp., Stillwell
Statistics: Dunnett's Test; * = $p \leq 0.05$; ** = $p \leq 0.01$

At 400 ppm, pup weights were statistically significantly lower than in the control group on days 4, 7, 14 and 21, for both generations, with the body weights ranging from 8–26 % below the control group. At 125 ppm, statistically significant lower pup weights were observed on days 7 and 14 for the F_1 pups and on days 7–21 for the F_2 pups. At 50 ppm, statistically significant lower pup body weights were observed in the F_2 group on days 4 and 7; pup body weights remained slightly below control values also on days 14 and 21.
Gross pathological findings:
There were no compound-related gross lesions in the F_1 or F_2 pups. Micropathology data was not collected for pups.
The increased incidence of splayed hind limbs observed in high-dose group dams during lactation was

probably due to the increase in food consumption, which caused the dose during the lactation phase to be approximately double the dose received during the premating and gestation phases.
The significantly decreased terminal body weights of 125-ppm group F_1 male rats obviously resulted primarily from body weight differences that were already present at weaning (bw on premating week 1 reduced by 8 % compared to controls); differences in body weight changes were minimal (3 %) between F_1 125 ppm males and control during the 11-week premating period (Table B.6.6-9).
From the results of this study, it could not be excluded that the statistically significantly decreased body weights of low-dose group F_2 pups on days 4 and 7 of lactation were treatment-related, although this was considered unlikely for the following reasons:
No significant effects were observed on days 14 and 21; F_2 pup weights at 50 ppm and 125 ppm during the first week of lactation were virtually the same, thus there was no obvious dose-response relationship; The pup body weights on days 4 and 7 were very close to historical control values.
For clarification of the significance of the findings at 50 ppm, a supplemental 2-generation reproduction study in rats was conducted, in which no reduction in F_1 or F_2 pup weights was seen (Eigenberg, 1997, TOX2001-1772).
The increased incidence of coarse tremors and the decreased pup body weight observed during the lactation phase (as early as lactation day 5 and ceased by lactation day 18 after weaning) in F_1 and F_2 pups at and above 125 ppm (19 and 59 mg/kg bw/d) occurred in the presence of maternal toxicity.
The excretion of cyfluthrin in rat milk has not been determined but it can be concluded that the presence of adverse effects in the offspring at 125 ppm was due to transfer of cyfluthrin or of its metabolite(s) in the milk during the lactation period. This conclusion is supported by the absence of adverse treatment effects on prenatal or perinatal litter parameters.

Conclusion:

Under the conditions of this two-generation reproductive toxicity study, cyfluthrin had no effect on fertility when administered via the diet to rats up to 400 ppm, the highest dose tested. The NOEL for parental toxicity was established at 50 ppm, based on reduced body weights of F_1 males at and above 125 ppm; at 400 ppm, clinical signs of neurotoxicity (splayed hind limbs) were observed in F_0 and F_1 females during lactation and body weights and food consumption were reduced in both sexes. The NOEL for offspring toxicity was established at 50 ppm, based on increased incidences of coarse tremors and decreased pup body weights at and above 125 ppm during the lactation period. It is not clear whether the presence of adverse effects in the offspring during lactation was due to transfer of cyfluthrin or of its metabolite(s) in the milk or a result of direct exposure of pups via the feed. For clarification, the extent of transfer of cyfluthrin (metabolites) via the milk should be investigated.

Re-evaluation by the RMS (2015):

400 ppm (26.7 mg/ kg bw/d):
Splaying of the hind limbs in F_0 and F_1 females during the lactation phase; Terminal body weights decreased in F_1 males (8 %) and females; Decreased body weights in F_0 females during the gestation phase (-13 % bw gain) and both F_0 and F_1 females during the lactation phase (30 % and 46 %); Decreased food consumption during the lactation period in F_0 and F_1 females; Coarse tremors in F_1 and F_2 pups (lactation day 5 until lactation day 18); Lower F_1 and F_2 pup weights on days 4, 7, 14 and 21.
125 ppm (8.3 mg/kg bw/d):
Terminal body weights decreased in F_1 males (6 %); Decreased food consumption during the lactation period in F_1 females; Coarse tremors F_1 and F_2 pups (lactation day 5 until lactation day 18); Lower F_1 and F_2 pup weights on days 4, 7, 14 and 21.
50 ppm (3.3 mg/ kg bw/d):
Lower F_2 pup weights on days 4 and 7; 14 and 21 (based on the supplemental study (Eigenberg D.A. (1997) this change is considered not to be due to compound administration)
NOAEL reproductive: 400 ppm
NOAEL offspring: 50 ppm based on coarse tremors in pups in the 125 and 400 ppm dose groups
NOAEL parental: 50 ppm

The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study is considered acceptable.

Cyfluthrin exposure through the milk is considered to be the main determinant of offspring neurotoxicity and it is proposed to classify beta-cyfluthrin as reproductive toxicant in category for effects via lactation. This proposal for classification was not made during the evaluation of the study for the addendum (2002, ASB2014-9599).
Classification and labelling for reproductive toxicity according to Regulation (EC) No 1272/2008 (GHS):
Lact H362: May cause harm to breast-fed children.

In a position paper submitted by the applicant an argumentation for the rebutting of this classification proposal is provided. In this statement it is agreed that the tremors seen in the early phase of lactation is attributed to exposure of the pups to cyfluthrin via the milk of the lactating parent females. It is further stated that these coarse tremors are transient and characteristic of acute neurotoxicity associated with Type II pyrethroids. Finally it is concluded that the age dependent sensitivity in young rats is consistent with a mode of action that is not relevant to infants and children, because it is a high dose phenomenom associated with a limited metabolic capacity of neonatal rats (in rats pyrethroids are primarly metabolised by cytochrome P450 enzymes and in humans by carboxylesterase enzymes) (Wason, 2014, ASB2014-7900).
However, the proposal for classification and labelling with Lact. H362 *'May cause harm to breast fed children'* is sustained by the RMS.

Data point:	KCA 5.6.1
Report:	[redacted], 1997, TOX2001-1772: A Supplementary Two-Generation Dietary Reproduction Study in Rats Using Technical Grade Cyfluthrin [redacted] Study-No. 94-672-CK, Report-No. 107474, Bayer File-No.: 8077, unpublished
Guideline(s):	OECD Test No. 416 (adopted January 2001)
Deviations:	Two instead of required three dose levels were tested.
GLP:	Yes
Acceptability:	Supplementary

(Experimental work from 10 January– 18 October 1995)

Materials and methods:

Test material: Technical grade cyfluthrin, purity: 94.6–96.2 %; batch no. 2030025
Test animals: Male and female Sprague-Dawley rats, age at study initiation: 7 weeks; Source: [redacted].
Technical grade cyfluthrin was administered at nominal dose levels of 0–25–50 ppm via the diet to Sprague-Dawley rats (30 rats/sex/group) for two generations (one mating per generation) to test for potential reproductive and neonatal effects. With three exceptions, material and methods applied in this supplemental 2-generation study fully corresponded to the 2-generation study by Eigenberg & Elcock (1996, TOX2001-1771):
Other dose levels were used; Rats were supplied by [redacted].; In the absence of clinical signs of neurotoxicity, the brain, spinal cord, and one sciatic nerve were not collected from all F_1 adults in the supplemental 2-generation study.

Results and discussions:

The intake of cyfluthrin, calculated using the analytical concentration of cyfluthrin in the feed is presented in Table B.6.6-12 below.

Table B.6.6-12: Rat 2-generation study: Test substance intake

Level	Mean doses in mg/kg bw/d				
	Males	Females			Males & Females
	Premating	Premating	Gestation	Lactation	Default calculation*
25 ppm	1.9	2.1	2.0	4.1	1.7
50 ppm	3.8	4.2	3.9	8.0	3.3

* based on default conversion factor of 15 proposed by JMPR (WHO) to be used for rat multi-generation studies

No compound-related clinical signs were observed in the adults. There were no compound-related mortalities. There was no compound-related effect on body weight or food consumption during the premating, gestation, or lactation periods. There were no compound-related effects on adult reproductive parameters. There were no compound-related effects on pup parameters. There were no compound-related gross or micropathological findings. No reproductive, neonatal, or parental toxicity was observed in this study.

Table B.6.6-13: Rat supplemental 2-generation study: Pup body weight development

Lactation day	Mean body weight of viable pups (g)					
	F_1 pups (males + females combined)			F_2 pups (males + females combined)		
	0 ppm	25 ppm	50 ppm	0 ppm	25 ppm	50 ppm
1	6.8	6.7	6.6	6.6	6.9	6.9
4[a]	10.2	10.2	10.0	9.9	10.6	10.3
4[b]	10.2	10.1	10.0	9.8	10.6	10.3
7	15.5	15.8	15.7	15.3	16.4	16.0
14	29.2	30.9	30.8	29.4	30.6	30.6
21	47.9	48.1	49.7	48.4	49.2	50.3

[a] before culling; [b] post culling

Conclusion:

No reproductive, neonatal or parental toxicity was observed in this supplemental study, which demonstrates that the statistically significant lower body weights of F_2 pups observed at 50 ppm at birth, an on lactation days 4 and 7 in the prior 2-generation reproduction study were not due to cyfluthrin administration. The NOEL for this study was 50 ppm, equivalent to 3.3 mg/kg bw/d.

Re-evaluation by the RMS (2015):

No compound-related changes were noted in the adults or in the offspring.
NOAEL reproductive, offspring, parental: 50 ppm (3.3 mg/kg bw/d)
The study is considered to be supplemental, since only a limited dose range (two dose levels) was tested. In the addendum to the monograph (2002, ASB2014-9599), this study was also considered as supplemental information.

B.6.6.2 Developmental toxicity studies

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

B.6.6.2.1 Oral study in rats

Data point:	KCA 5.6.2
Report:	[redacted], 1982, TOX9401908 FCR 1272 - Evaluation for Embryotoxic and Teratogenic Effects on Orally Dosed Rats. Report no.: 10562 (January 20, 1982); [redacted]
Guideline(s):	The study was performed partly to the OECD-Guideline 414 which complies to Directive 87/302/EEC, Part B.
Deviations:	Mating was performed with one male and two females; treatment from days 6 (not 5) through 15 of gestation; food consumption, number of corpora lutea and weight of uterus was not reported; individual foetal data and incidence tables were not given in the report.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Acceptable

(Dates of exp. work: October 19, 1979 - November 27, 1979)

Materials and methods:

Groups of 25 inseminated rats (BAY:FB 30; source: [redacted]) were treated with cyfluthrin (batch no: 16001/79; purity: approx. 85 %) in daily oral doses of 0, 3, 10, and 30 mg/kg bw from the 6th to the 15th day of gestation. The vehicle was polyethylene glycol E 400.
Statistical methods: U-test of Wilcoxon-Mann-Whitney, chi-square test (modification by Yates), Fishers "exact test".

Results and discussions:

A high-stepping gait, occasionally ataxia and reduced motility were observed in a few dams after administration of the mid- and high-dose (10 and 30 mg/kg bw/d). Doses up to 30 mg/kg bw had no lethal effect and did not affect average weight gain.

Table B.6.6-14: General examinations (parental data)

Dose [mg/kg bw]	0	3	10	30
No of inseminated rats	25	25	25	25
No of pregnant rats	25	23	25	22
No of implantations	12.1	12.2	11.3	11.5
No of live fetuses	11.1	11.4	10.3	10.5
Mean fetal weight [g]	4.09	4.26	4.39**	4.29*

* = p <0.05, ** = p <0.01.

The increase of mean foetus weight was not dose related. No embryotoxic and/or teratogenic effects were observed. Malformations were of a comparable type and frequency in all groups.

Table B.6.6-15: Anomalies (% foetuses)

Dose [mg/kg bw]	0	3	10	30
Hypoplasia of telencephalon	2.88	0	0	0
Wavy ribs	0.04	0	0	0
Microphthalmia, anophthalmia, hydrocephalus	0.04	0	0	0
Monster syndrome	0	0.04	0	0
Cryptorchidism	0	0	0.04	0
Malformations of extremities	0	0	0	0.09

Conclusion:

The NOEL of 3 mg/kg bw/d for maternal toxicity and of 30 mg/kg bw/d for foetotoxicity was based on clinical signs in dams at 10 and 30 mg/kg bw/d and the lack of embryotoxic or teratogenic effects on foetuses at 30 mg/kg bw/d.

Re-evaluation by the RMS (2015):

30 mg/kg bw/d:
High-stepping gait, ataxia and reduced motility observed in a few dams.
10 mg/kg bw/d:
High-stepping gait, ataxia and reduced motility observed in a few dams.
3 mg/kg bw/d:
No effect.
NOAEL maternal toxicity: 3 mg/kg bw/d
NOAEL embryotoxic: ≥30 mg/kg bw/d
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.6.2
Report:	[redacted] 1983, TOX9401909: Embryotoxicity (including teratogenicity) study with FCR 1272 in the rat. Report no.: R2774 (December 14, 1983); [redacted]
Guideline(s):	The study was performed principally according to the OECD-Guideline 414 which complies to Directive 87/302/EEC, Part B.
Deviations:	Treatment from days 6 (not 5) through 15 of gestation; individual clinical data and percent of corpora lutea were not reported. 1/3 of foetuses were used for visceral examination and 2/3 for skeletal examinations.
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(Dates of exp. work: September - October 1983).

Materials and methods:

Groups of 25 inseminated Wistar rats (KFM-HAN; source: [redacted]) were given daily oral cyfluthrin doses of 0, 1, 3, and 10 mg/kg bw from the 6th to the 15th day of gestation. The dispersion of the test article (batch no: 816170019, purity: 93.4 %) in

Cremophor EL/distilled water (1 % v/v) was prepared daily short before application.
Statistical methods: Mean values, standard deviations with appropriate methods (not given), sex ratio of foetuses with chi-square test.

Results and discussions:

The animals on 10 mg/kg bw ate less than the controls for the first 6 days of treatment. Otherwise none of the parameters studied exhibited any evidence of effects on the dams or on foetal development that could be attributed to the treatment.

Table B.6.6-16: General examinations (parental data)

Dose [mg/kg bw]	0	1	3	10
Inseminated rats	25	25	25	25
Pregnant rats	25	25	21	25
Number of implantations	11.0	11.0	11.5	12.0
Number of live fetuses	10.2	10.7	11.0	11.7
Mean weight of fetuses [g]	4.9	5.0	5.0	4.9

Table B.6.6-17: Anomalies (% foetuses)

Dose [mg/kg bw]	0	1	3	10
Absent sternebrae	1.2	1.7	0	1.6
Coagulated blood in the abdominal cavity	1.2	1.1	0	1.1
Coagulated blood in the pelvis left kidney	0	0	0	1.1
Abnormally-shaped ribs	0.6	0	0	0
Longitudinally-split sternebrae	0	0.6	0	0

No embryotoxic or teratogenic effects were observed.

Conclusion:

The NOEL of 10 mg/kg bw/d for maternal and foetotoxicity was based on the absence of effects in dams and foetuses at this dose.

Re-evaluation by the RMS (2015):

The NOAEL of 10 mg/kg bw/d for maternal and foetotoxicity was based on the absence of effects in dams and foetuses at this dose.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered acceptable.

B.6.6.2.2 Oral study in rabbits

Data point: KCA 5.6.2

Report: [redacted] 1983, TOX9401914:
FCR 1272 - Study for embryotoxic effects on rabbits after oral administration. Report no.: 11855 (June 1, 1983); [redacted]

Guideline(s): The study was performed partly to the OECD-Guideline 414 which com-

	plies to Directive 87/302/EEC, part B.
Deviations:	Individual foetal data and incidence tables not given; food consumption data not reported, gravid uterus not weighed, number of corpora lutea not reported.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Acceptable

(Dates of exp. work: April - July 1982)

Materials and methods:

Groups of 15 inseminated Himalayan rabbits (CHBB:HM; source: [redacted]) received cyfluthrin (batch no. 816170019, purity: 95.0 %) in daily oral doses of 0, 5, 15 or 45 mg/kg bw from the 6th to the 18th day of gestation. Cremophor EL/water (0.5 %) served as formulation agent.
Statistics: U-test of Wilcoxon-Mann-Whitney, chi-square test (modification by Yates), Fishers "exact test".

Results and discussions:

After daily treatment with 45 mg/kg bw/d two dams aborted and one dam resorbed its implants completely. These events were interpreted as probable maternal-toxic effects. No further clinical findings or changes on body weights and no signs of embryotoxic or teratogenic potential of the test substance were recorded in any of the groups.

Conclusion:

The NOEL of 15 mg/kg bw/d for maternal toxicity and of 45 mg/kg bw/d for foetotoxicity was based on miscarriage of dams at 45 mg/kg bw/d and on the absence of foetotoxic effects at doses up to 45 mg/kg bw/d.

Re-evaluation by the RMS (2015):

45 mg/kg bw/d:
Abortion (two dams), resorption of implants (one dam)
15 mg/kg bw/d/5 mg/kg bw/d:
None
NOAEL maternal: 15 mg/kg bw/d
NOAEL developmental: 45 mg/kg bw/d
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.6.2
Report:	[redacted], 1992, TOX9401915: Embryotoxicity study (including teratogenicity) with FCR 1272 in the rabbit. Report no.: R 5770 (December 03, 1992); [redacted]
Guideline(s):	The test was conducted according to the OECD Guideline no. 414 which complies to Directive 87/303/EEC, part B (teratogenicity).
Deviations:	None that compromised the validity of the study results
GLP:	The test followed the OECD principles of GLP (declaration of testing facility)
Acceptability:	Acceptable

(Dates of exp. work: February 18, 1992 - March 31, 1992)

Materials and methods:

Groups of 16 inseminated Chinchilla rabbits (CHbb: CH Hybrids, source: [redacted]) were treated orally by gavage with cyfluthrin (batch no.: 2380051769, purity 96.0 %, formulated in corn oil) in doses of 0, 20, 60, and 180 mg/kg bw from the 6th to the 18th day of gestation.
Statistical methods: Univariate one-way analysis of variance, Dunett-test, Steel-test, Fishers exact test.

Results and discussions:

The evaluation of the food consumption data resulted in a dose-related reduced mean food consumption during the treatment period at 60 and 180 mg/kg bw. In these two groups, statistically significantly increased mean food consumption was noted during the last recording period (24.-28. day). This finding was considered to be a compensatory reaction to the previous reduction in food consumption.
The development of the mean body weight correlated with the reduced food consumption and showed a dose-related, statistically significant body weight loss in group 3 (60 mg/kg bw) and group 4 (180 mg/kg bw) during the treatment period. The corrected body weight gain has not shown any changes, related to the substance administration.
No deaths ensued. No deviations from the physiological norm were revealed by clinical observation and at necropsy.

Table B.6.6-18: General examinations (parental data)

Dose: [mg/kg bw]	0	20	60	180
Food intake (%, 6.-11. d) Signi	100	-15.1	-26.7 *	-47.9 **
Food intake (%, 24.-28. d) Signi	100	+20.7	+33.1 **	+47.1 **
Weight gain [%] (6.-19. d) Signi	-0.9	-0.8	-4.6 **	-5.6 **
Weight gain [%] (6.-28. d) Signi	2.1	3.4	1.0	-0.1
Corrected weight gain [g] Signi	-9.9	-7.3	-9.8	-10.9

* = $p < 0.05$, ** = $p < 0.01$.

In the lowest dose group of 20 mg/kg bw a reduced number of pregnant rabbits and a decreased number of implantation sites were observed. Since no dose-relation could be established, this finding is considered incidental in nature and without toxicological relevance.
From 60 mg/kg bw an increase in the number of post-implantative resorptions was the only observed change interpretable as a sign of reproduction toxicity. In consequence, the number of foetuses in percentage of implantation sites was reduced.
Determination of the foetal weight and the foetal sex ratio as well as the external and visceral inspection of the foetuses yielded no evidence of embryotoxic or teratogenic effects.

Table B.6.6-19: General examinations (reproduction data)

Dose: [mg/kg bw]	0	20	60	180
Number of pregnant dams	16	13	16	15
Corpora lutea	201	141	194	189

Implantation sites Signi	193	128 *	183	186
Post-implantation loss Signi	21	14	36 *	53 **
Embryonic resorptions Signi	7	8	21 **	28 **
Total fetuses % of implant. sites Signi	172 89.1	114 89.1	147 80.3 *	133 71.5 **

* = p <0.05,** = p <0.01.

Conclusion:

The NOEL of 20 mg/kg bw/d for parental toxicity was based on decreased food consumption and body weight gain during the treatment period. The NOEL of 20 mg/kg bw/d for foetotoxicity was based on increased post-implantative resorptions at 60 mg/kg bw/d and above.

Re-evaluation by the RMS (2015):

180 mg/kg bw/d:
reduced mean food consumption during the treatment period; body weight loss during the treatment period; increase in the number of post-implantative resorptions.
60 mg/kg bw/d:
reduced mean food consumption during the treatment period; body weight loss during the treatment period; increase in the number of post-implantative resorptions.
20 mg/kg bw/d:
None
NOAEL maternal: 20 mg/kg bw/d
NOAEL developmental: 20 mg/kg bw/d
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October 1996 (ASB2010-10436), the study was considered acceptable.

B.6.6.2.3 Inhalation studies in rats

Data point:	KCA 5.6.2
Report:	[redacted], 1988, TOX9401910: FCR 1272 - Study for embryotoxic effects on rats after inhalation. Report no.: 16391 (February 01, 1988, report), 16391A (August 16, 1988, addendum); [redacted]
Guideline(s):	The teratogenicity part complies to a great extend to OECD-Guideline 414 and to Directive 87/302/EEC, part B. The inhalation part was conducted according to OECD-Guideline no. 412 which complies to Directive 92/69 EEC method B 8.
Deviations:	None that compromised the validity of the study results (the total number of pups per dose group was not given)
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(Dates of exp. work: July - August 1985, January - February 1986).

Materials and methods:

Two separate experiments were performed in which groups of 30 inseminated female rats (Bor:WISW (SPFCpb); source: [redacted]) received cyfluthrin (1st exp. - batch no.: 233490583, purity: 92.9-93 %; 2nd exp. - batch no.: 238005176, purity 96.2 % formulated in ethanol/polyethylene glycol E 400 as aerosol; head-nose exposure). The nominal concentrations were 0 (vehicle), 1, 5, 25 mg/m³ air in the 1st experiment and 0 (vehicle), 0.42, 1.4 and 3.4 mg/m³ air in the 2nd experiment. These corresponded to analytical concentrations of 1.1, 4.7, and 23.7 mg/m³ air and 0.09, 0.25, and 0.59 mg/m³ air, respectively. The head-nose exposure was from the 6th to the 15th day of gestation for 6 h per day under dynamic conditions. Oxygen substitution (30 % O_2) was carried out in a further group exposed to a nominal concentration of 5 mg/m³ (analytical concentration 4.16 mg/m³ air).
Statistical methods: Wilcoxon-Mann-Whitney-U-test; Chi square test (modified by Yates); Fishers exact test.

Results and discussions:

The analysis of the test atmosphere showed that stable and reproducible conditions of exposure were present during the study. About 90 % of the aerosol mass may be regarded as readily respirable (particles <5 µm).

Table B.6.6-20: General examinations (parental data, experiment 1)

Dose [mg/m³ air]	0	1.1	4.7	23.7
Number of inseminated rats	30	30	30	30
Number of pregnant rats	25	29	27	29
Number of implantations	11.5	12.2	11.7	11.6
Weight gain during pregnancy [g]	75.5	66.6*	57.1**	45.6**
Number of live fetuses	10.8	11.3	10.1	9.3
Mean weight of fetuses [g]	3.4	3.16*	2.89**	2.43**
Mean weight of placenta [g]	0.57	0.52*	0.48**	0.40**

* = p <0.05, ** = p <0.01.

In the dams no deaths occurred as a result of the treatment. At 4.16 mg/m³ air (+O_2) and from 4.7 mg/m³ air onwards reduced motility, piloerection, ruffled, unkempt fur, irritation of the visible eye mucous membranes and laboured breathing were observed. The rats with oxygen substitution tolerated the exposure better (lower intensity) than the corresponding rats without the oxygen exposure.
Body weight development of dams was reduced from the dose of 1.1 mg/m³ air both during the administration and the remaining gestation period. At 4.16 mg/m³ air with oxygen substitution the body weight development was retarded only during the administration period. Both, clinical signs and the decreased body weight gain were interpreted as an indication of maternal toxicity. At 1.1 mg/m³ air onwards mean foetus and placenta weights were lower, the number of runts higher.

Table B.6.6-21: General examinations (parental data, experiment 2)

Dose [mg/m³ air]	0	0.09	0.25	0.59	O_2+4.16
Number of inseminated rats	30	30	30	30	30
Number of pregnant rats	23	29	25	29	22
Number of implantations	10.7	11.4	11.2	11.0	11.2
Weight gain during pregnancy [g]	58.4	63.0	60.2	85.9	56.4

Number of live fetuses	9.0	9.6	8.8	9.2	9.5
Mean weight of fetuses [g]	3.48	3.51	3.53	3.47	3.29*
Mean weight of placenta [g]	0.61	0.61	0.62	0.58	0.56*

* = p <0.05, ** = p <0.01.

The slightly increased frequency of microphthalmia (unilateral) at 23.7 mg/m³ air was outside the historical control values (6 incidences in 8 studies in 1984, 2 incidences in 15 studies in 1985) for this finding. It is questionable whether this malformation was attributed to cyfluthrin with head-nose exposure or due to the hypoxic status of the dams provoked by the reflex bradypnoea and hypothermia. No further evidence of a teratogenic potential was found at doses up to and including the highest, clearly maternal-toxic dose.

Table B.6.6-22: Anomalies (mean values / standard deviation)

Dose [mg/m³air]	0	1.1	4.7	23.7
Skeletal variations	1.80 / 171	2.62 / 1.59	3.89* / 2.47	5.32** / 2.65
Runts	0.20 / 0.50	2.00* / 3.13	4.89** / 4.64	7.57** / 4.15
Malformations (all)	0.04 / 0.20	0.07 / 0.26	0.15 / 0.46	0.29 / 0.71
Microphthalmia: absolute number of pups	1/271	2/319	2/292	8/261

* = p <0.05, ** = p <0.01.

In addition, from the dose of 1.1 mg/m³ air onwards reduced foetal weights and a higher number of foetuses with retarded ossification were observed. In addition at 23.7 mg/m³ air an increased incidence of resorptions occurred.
These effects were interpreted as signs of a non-specific retardation of embryonic development and are attributed to a maternal hypoxia induced by the treatment rather to an embryotoxic potential of cyfluthrin (see also “Mechanistic studies”). Accordingly, the effects were considerably less pronounced at 4.16 mg/m³ air with oxygen substitution than at 4.7 mg/m³ air without oxygen substitution.
The data of the addendum provide explanations for the reproductive effects observed. Accordingly, the reflex bradypnoea of the dams which is compensated by hypothermia and a reduction in metabolic activity seems responsible for the impairment of intra-uterine processes.

Table B.6.6-23: Anomalies (mean values / standard deviation)

Dose [mg/m³ air]	0	0.09	0.25	0.59	O_2+4.16
Skeletal variations	2.52/2.19	2.45/1.92	1.64/1.41	1.86/1.77	2.82/1.30
Runts	0.35/0.78	0.38/0.73	0.32/0.69	0.21/0.49	1.14*/1.58
Malformations (all)	0.04/0.21	0.10/0.31	0.20/0.65	0.03/1.19	0.05/0.21
Microphthalmia: absolute number of pups	1/206	1/278	2/221	1/268	1/209

* = p <0.05, ** = p <0.01.

Conclusion:

The NOEL of 0.59 mg/m³ air for maternal toxicity and foetotoxicity was based on reduced body weight of dams exposed to 1.1 mg/m³ air and on reduced foetal weight and retarded ossification of foetuses at the same concentration.

Re-evaluation by the RMS (2015):

23.7 mg/m³ air:
Clinical signs of the dams (reduced motility, piloerection, ruffled, unkempt fur, irritation of the visible eye mucous membranes and laboured breathing); Reduced body weight development; Lower mean foetus and placenta weights; Higher number of runts; Reduced foetal weights and a higher number of foetuses with retarded ossification; Increased incidence of resorptions; Slighty increased frequency of microphthalmia (unilateral).
4.7 mg/m³ air:
Clinical signs of the dams (reduced motility, piloerection, ruffled, unkempt fur, irritation of the visible eye mucous membranes and laboured breathing); Reduced body weight development; Lower mean foetus and placenta weights; Higher number of runts; Reduced foetal weights and a higher number of foetuses with retarded ossification.
O_2 + 4.16 mg/m³ air:
Clinical signs of the dams (reduced motility, piloerection, ruffled, unkempt fur, irritation of the visible eye mucous membranes and laboured breathing); Reduced body weight development;
Lower mean foetus and placenta weights; Higher number of runts; Reduced foetal weights and a higher number of foetuses with retarded ossification.
1.1 mg/m³ air:
Reduced body weight development; Lower mean foetus and placenta weights; Higher number of runts; Reduced foetal weights and a higher number of foetuses with retarded ossification.
0.59 mg/m³ air/0.25 mg/m³ air/0.09 mg/m³ air:
None
NOAEL maternal: 0.59 mg/m³ air
NOAEL developmental: 0.59 mg/m³ air
The slightly increased frequency of microphthalmia (unilateral) at 23.7 mg cyfluthrin/m³ air was considered to represent a secondary effect due to hypoxic conditions in the dams. Due to the irritating properties of the test substance at the highest dose of 23.7 mg/m³ air a reflex bradypnoea occurred in the dams which was compensated by hypothermia and a reduction in metabolic activity. It can be assumed that the occurrence of microphthalmia in the offspring does not represent a direct toxic effect of the test substance. This assumption is supported by reproductive toxicity studies with orally administered cyfluthrin/beta-cyfluthrin where no treatment-related malformations were observed. It is therefore proposed not to classify cyfluthrin for embryotoxic effects in the presence of maternal toxicity (cat. 2). Anyhow, it is proposed to classify and label cyfluthrin/beta-cyfluthrin according to the respiratory irritating effects in human volunteers (STOT-SE 3 H335 May cause respiratory irritation / Xi; R37 Irritating to respiratory system).
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Data point:	KCA 5.6.2
Report:	[redacted], 1993, TOX9401829: FCR 1272 (c.n. Cyfluthrin), Inhalation study for embryotoxic effects in rats. Report no.: 22581(October 05, 1993); T 3041008 (October 5, 1993, addendum); [redacted]
Guideline(s):	The test was conducted according to OECD Guideline no. 414 which conforms to Directive 87/303/EEC, Part B. Technically, the inhalation was conducted according to the OECD Guideline no. 412 which complies to Directive 92/69 EEC method B 8
Deviations:	None that compromised the validity of the study results (mating was performed with one male and two females in on cage)

GLP: Yes

Acceptability: Acceptable

(Dates of exp. work: March - April 07, 1993).

Materials and methods:

Main groups of 25 inseminated Wistar rats (Bor:WISW (SPFCpb); source: [redacted]) received cyfluthrin (batch no.: 238005176, purity 96.2 %, 94.7 %, 94.9 % (successive determinations)). The test substance was formulated in ethanol/polyethylene glycol E 400. The rats were exposed head/nose only under dynamic conditions 6 h per day from day 6 to 15 of gestation. The nominal concentrations were 0 (air), 0 (vehicle), 0.5, 2.5, 12.5 mg/m³ air corresponding to analytical concentrations of 0.46, 2.55, 11.9 mg/m³ air. An additional group was exposed to 12.5 mg/m³ air (analytical concentration 12.8 mg/m³ air) supplemented with 40 % oxygen.
For every concentration a satellite group with 5 pregnant rats was established and exposed for eight days (day 0 to day 7 corresponding to day 6 to 13 of gestation). In this group parameters of maternal toxicity (including some specific parameters) were determined: Mortality, clinical signs, body weight and food intake day 0 to day 7, lung function parameters day 0, reflexes and rectal temperature day 0 and day 6, plasma levels of cyfluthrin (see Schmidt, 1993, TOX9401913) and pathological examination day 7.
Lung function tests: Five pregnant rats per dose (satellite animals) were exposed to cyfluthrin in a plethysmograph for 4-5 h. To achieve a total exposure time of 6 h the rats were exposed thereafter in the ″normal″ head-nose only inhalation chamber. The following lung function parameters were evaluated: Peak expiratory flow, tidal volume, breaths per minute, respiratory minute volume, inspiratory time and expiratory time. The foetuses were delivered by cesarian section on the 20th day of gestation.
Statistical methods: Fishers "exact test", F-test, t-test, chi-square test (modification by Yates), ANOVA-test-set (for the special investigations) special tests (for characterisation of the aerosol distribution).

Results and discussions:

Stable and reproducible conditions of exposure were achieved. The aerosol had a mean mass media aerodynamic diameter (MMAD) of about 1.1 µm. More than 98 % of the aerosol mass may be regarded as readily respirable (particles <3 µm).
In the dams of the main group, food intake and body weight development were decreased at levels of 0.46 mg/m³ air and above (Table B.6.6-24). Clinical signs (bloody snout, unkempt fur and piloerection) were apparent in the dams at 2.55 mg/m³ air and above. Respiratory disturbances and hypoactivity were noted at 11.9 mg/m³ air and 12.8 mg/m³ air (plus oxygen), and a high-stepping gait and salivation at 11.9 mg/m³ air only. No gross pathological findings were recorded at necropsy of any dose group (including the satellite groups).

Table B.6.6-24: General examinations (parental data)

Dose [mg/m³ air]	0 a.	0 v.	0.46	2.55	11.9	O2+12.8
Number of inseminated rats	25	25	25	25	25	25
Dams with viable fetuses	21	22	23	23	23	23
Number of implantations	12.3	12.8	11.3	11.4	11.3	11.3
Food intake, pregnancy	19.9	20.0	19.1**	18.7**	17.7**	17.4**
Weight gain, pregnancy [g]	83.6	88.8	76.8	74.7**	58.7**	62.3**
Corrected weight gain [g]	20.0	23.0	19.8	19.3*	13.6**	12.5**

Number of live fetuses	11.6	12.0	10.7	10.9	10.4*	10.4*
Mean weight of fetuses [g]	3.41	3.50	3.48	3.13**	2.48**	2.83**
Mean placenta weight [g]	0.61	0.60	0.62	0.56*	0.46**	0.51**

a = air control, v = vehicle control; * = p <0.05, ** = p <0.01 in relation to air and vehicle control.

Placental weights were lower from 2.55 mg/m³ air onwards and foetuses showed signs of retarded development (reduction of foetal weight).
At 2.55 mg/m³ air and above, foetuses exhibited signs of retarded ossification of the phalanges, metacarpals and metatarsals (except in the 2.55 mg/m³ group), sternebrae, vertebrae, pelvis or the skull. Statistically significant instances of retarded ossification, which were less frequent in the 2.55 mg/m³ group than in either of the high dose groups, were evident in most cases when the calculations were made on individual foetal or litter basis. With oxygen supplement the embryotoxic findings in the high dose group were less pronounced.
An increased incidence of malformations was also observed at levels of 2.55 mg/m³ air and above (Table B.6.6-25). With the exception of the occurrence of microphthalmia and anophthalmia in the high dose groups, the nature of malformations were comparable to those in the controls of this or previous studies (hydrocephalus internus: 1/0/0/0/0/0; skeletal dysplasia of legs: 0/1/1/4/1/3; filiform tail: 0/0/0/1/0/0; spinal malformation: 0/0/0/0/2/0; rib malformation: 0/0/0/0/1/0; malformation of exoccipital bone and cervical vertebral arches: 1/0/0/0/3/0; dysplasia of exoccipital bone: 0/0/0/0/1/0; umbilical hernia: 0/0/0/0/1/0) did not indicate a specific teratogenic potential of cyfluthrin inhalation. The incidence of microphthalmia was outside the historical control values (1983-1984).

Table B.6.6-25: Malformations

Dose [mg/m³ air]	0 a.	0 v.	0.46	2.55	11.9	O_2+12.8
Microphthalmia (Fetuses / Litter affected)	1/1	2/2	1/1	3/2	13/8**	7/5
Anophthalmia (Fetuses / Litter affected)	-	-		-	1/1	1/1
Fetuses per group (n)	243	263	245	251	239	240
Total malformed fetuses (n)	3	3	2	8	21***	10
Litters with malformations (n)	2	3	2	4	10*	7

* = p <0.05, ** = p <0.01, *** = p <0.001

In the addendum, inhalation specific details are reported (results of the lung function tests, rectal temperature and plasma concentrations).
The satellite groups exhibited a concentration-dependent hypothermia and bradypnoea (hypoventilation) after the 1st exposure to levels of 0.46 mg/m³ air and above. After the seventh exposure this hypothermia could still be determined in the high dose groups only, being less severe in the group with oxygen substitution. In the satellite groups concentrations up to 2.55 mg/m³ air were tolerated without an effect on body weight gain. No signs of toxicologically significant neurological or sensorimotor changes (reflex tests) were seen. Comparing the findings from the groups with and without oxygen substitution permits the conclusion that the increase in the partial pressure of oxygen in the inhalation chamber produced an attenuation of the maternal toxic effects. There were no significant differences in the plasma cyfluthrin levels in the groups with and without oxygen substitution.

Conclusion:

The NOAEL of 0.46 mg/m³ air for maternal toxicity and the NOEL of 0.46 mg/m³ air for foetotoxicity was based on reduced food consumption and body weight development of dams during pregnancy and on reduced placental weights and retardation of development. For maternal toxicity a NOAEL was established, because transient, marginal changes (reduced food intake during pregnancy in the main group, hypothermia and reflexively induced bradypnea in the satellite group) were already observed at

this dose. However these transient effects were not regarded as toxicologically relevant. The embryotoxicity of cyfluthrin after inhalative exposure is considered to be caused by a physiological maternal compensation mechanism (hypothermia with respiratory alkalosis) following reflex bradypnoea after sensory irritation.

Re-evaluation by the RMS (2015):

O_2+12.8 mg/m³ air:
Decreased food intake and body weight development in dams; Clinical signs (bloody snout, unkempt fur and piloerection) in dams; Respiratory disturbances and hypoactivity in dams; Lower placental weights; Retarded development in foetuses (reduction of weight); Retarded ossification of the phalanges, metacarpals and metatarsals, sternebrae, vertebrae, pelvis or the skull; Higher incidence of microphthalmia and anophthalmia; Concentration-dependent hypothermia and bradypnoea (hypoventilation) in dams (lung function tests).
11.9 mg/m³ air:
Decreased food intake and body weight development in dams; Clinical signs (bloody snout, unkempt fur and piloerection) in dams; Respiratory disturbances and hypoactivity in dams; High-stepping gait and salivation in dams; Lower placental weights; Retarded development in foetuses (reduction of weight); Retarded ossification of the phalanges, metacarpals and metatarsals, sternebrae, vertebrae, pelvis or the skull; Higher incidence of microphthalmia and anophthalmia; Concentration-dependent hypothermia and bradypnoea (hypoventilation) in dams (lung function tests).
2.55 mg/m³ air:
Decreased food intake and body weight development in dams; Clinical signs (bloody snout, unkempt fur and piloerection) in dams; Lower placental weights; Retarded development of foetuses (reduction of weight); Retarded ossification of the phalanges and metacarpals, sternebrae, vertebrae, pelvis or the skull; Concentration-dependent hypothermia and bradypnoea (hypoventilation) in dams (lung function tests).
0.46 mg/m³ air:
Decreased food intake and body weight development in dams; Concentration-dependent hypothermia and bradypnoea (hypoventilation) in dams (lung function tests).
LOAEC maternal: 0.46 mg/m³ air
NOAEC maternal <0.46 mg/m³ air
LOAEC (developmental): 2.55 µg/L
NOAEC developmental: 0.46 mg/m³ air

The maternal NOAEC is deviating from the evaluation in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), where a NOAEL of 0.46 mg/m³ air is proposed. At this dose level a decreased food intake and body weight development in dams, as well as a concentration-dependent hypothermia and bradypnoea (hypoventilation) in dams (lung function tests) was noted. Contrary to the evaluation in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the changes were considered toxicologically relevant. Likewise, the increased frequency of malformations (microphthalmia, anophthalmia, bone malformations) in the offspring needs to be discussed more in detail. As discussed in the previous study of [redacted] (1988, TOX9401910), the increased frequency of microphthalmia at 11.9 and at 12.8 mg cyfluthrin/m³ air with oxygen supplement was considered to represent a secondary effect due to hypoxic conditions in the dams. Due to the irritating properties of the test substance at these dose levels a reflex bradypnoea occurred in the dams which was compensated by hypothermia and a reduction in metabolic activity. It can be assumed that the occurrence of the mentioned malformations in the offspring does not represent a primary toxic effect of the test substance. This assumption is supported by reproductive toxicity studies with orally administered cyfluthrin/beta-cyfluthrin where no treatment-related malformations were observed. It is therefore proposed not to classify cyfluthrin for embryotoxic effects in the presence of maternal toxicity (cat. 2). Anyhow, it is proposed to classify and label cyfluthrin/beta-cyfluthrin according to the respiratory irritating effects (STOT-SE 3 H335 May cause respiratory irritation / Xi; R37 Irritating to respiratory system).
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

B.6.6.2.4 Determination of plasma concentration

Data point:	KCA 5.6.2
Report:	[redacted] 1993, TOX9401913: FCR 1272, Determination of the FCR 1272 concentration in the plasma of rats following inhalative exposure - Report no.: 22726 (December 02, 1993); [redacted]
Guideline(s):	No (no guidelines available)
Deviations:	Not applicable
GLP:	No (analytical study)
Acceptability:	Acceptable

(Dates of exp. work: June 1992).

Materials and methods:

This study was part of the inhalation study for embryotoxic effects in rats and the experiment design is described under [redacted], 1993, TOX9401829.
From five pregnant anesthetised rats of exposure groups air control, vehicle control, 0.5, 2.5, 12.5 and 12.5 + O_2 mg/m³ air blood was sampled by cardiac puncture after the last exposure (7th day) with cyfluthrin (batch no.: 380267024, purity 92 %) on day 7. The test substance was first dissolved in 5 mL 1,4-dioxane and this solution made up to 50 mL with n-hexane in a volumetric flask to prepare a 10 mmole/L stock solution. The cyfluthrin content in the plasma was determined with a gas-chromatographic method using a nickel detector (^{63}Ni ECD).

Results and discussions:

Very low concentrations of cyfluthrin were found in the plasma. Definitely detectable amounts of cyfluthrin were only found in the high-dose groups 12.5 mg/m³ air and 12.5 mg/m³ air (+39 % oxygen). Since cyfluthrin is apparently strongly bound to plasma proteins, the substance is incompletely recovered from the plasma samples. The objective of this study was to examine the question as to whether substitution of the inhaled air by oxygen would affect the concentration of cyfluthrin in the blood.

Conclusion:

The concentrations of cyfluthrin were low (19 + 13.3 pmole/mL and 14.7 + 4.4 pmole/mL, respectively) and were not affected by oxygen supplementation.

Re-evaluation by the RMS (2015):

Very low concentrations of cyfluthrin were found in the plasma. Definitely detectable amounts of cyfluthrin were only found in the high-dose groups 12.5 mg/m³ air and 12.5 mg/m³ air (+39 % oxygen).
The study provides additional information and is considered acceptable under the conditions of the study and based on the information given in the report. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable as supplemental information.

Studies evaluated in the addendum 1 to the monograph (2002, ASB2014-9599):

Data point:	KCA 5.6.2
Report:	[redacted] 1996, TOX2001-1773: A Developmental Toxicity Study with FCR 4545 Technical in the Wistar Rat [redacted] Study-No. 95-612-EW, Report-No. 107453, Bayer File-No.: 7989, unpublished (Experimental work from 13 November – 13 December 1995)
Guideline(s):	OECD Guideline No. 414 (adopted January 2001)
Deviations:	None that compromised the validity of the study results (treatment from days 6 (not 5) through 15)
GLP:	Yes
Acceptability:	Acceptable

Materials and methods:

Test material: Beta-cyfluthrin technical ("FCR 4545 Technical"), purity: 96.5–97.3 %, batch-no.: 3030125, suspended in 1 % aqueous Cremophor
Test animals: Female Wistar rats, age at start of treatment: approx. 12–15 weeks; Source: [redacted]
20 sperm-positive female Wistar rats/group were administered nominal doses of 0–3–10–40 mg beta-cyfluthrin/kg bw/d by oral gavage on days 6 through 15 of gestation. Maternal toxicity, as demonstrated by clinical signs and changes in body weight gain and food consumption during gestation, was characterised. All dams were sacrificed on gestation day 20, at which time the foetuses were removed by caesarean section and a gross maternal necropsy was performed. All foetuses were sexed, weighed, and evaluated for external anomalies. Approx. half of each litter was examined for visceral effects; the other half underwent a skeletal examination.

Results and discussions:

Results maternal toxicity:
Clinical findings:
Clinical findings in dams was confined to the high-dose group, where increased incidence of mortality, hypoactivity, locomotor incoordination, and salivation were found.

Table B.6.6-26: Rat developmental toxicity: Clinical signs and mortality

Clinical findings	Incidence of clinical findings during gestation days 6–15 in the dose groups			
	0 mg/kg bw/d	3 mg/kg bw/d	10 mg/kg bw/d	40 mg/kg bw/d
Mortality	0	0	0	3
Hypoactivity	0/27	0/24	0/21	26/26
Locomotor incoordination	0/27	0/24	0/21	26/26
Salivation	0/27	0/24	0/21	25/26

Body weight and food consumption:
Statistically significantly decreased body weight gain of dams were observed at 40 mg/kg bw/d (see next table). In the 10 mg/kg bw/d dose group, evidence of toxicity was limited to slightly decreased body weight gain during the period of beta-cyfluthrin gavage administration, which reached statistical

significance during gestational day 7–8. Statistically significantly decreased food consumption that was considered treatment-related was observed in the mid- and high-dose group.

Table B.6.6-27: Rat developmental toxicity: Body weight gain and food consumption

Mean body weight gain (g)				
Treatment period	Dose group (mg/kg bw/d)			
	0	3	10	40
Day 6–16	38.4 (100 %)	36.3 (95 %)	34.8 (91 %)	17.2** (45 %)
Day 0–20	100.6 (100 %)	98.0 (97 %)	98.2 (98 %)	83.6** (83 %)
Net body weight changes	44.9 (100 %)	40.1 (89 %)	38.5* (86 %)	30.5** (68 %)
Mean food consumption (g/kg bw/d)b				
Treatment period	Dose group (mg/kg bw/d)			
	0	3	10	40
Day 6–16	86.3 (100 %)	82.1 (95 %)	75.8 (88 %)	60.0 (70 %)
Day 0–20	85.4 (100 %)	82.5 (97 %)	78.6 (92 %)	74.0 (87 %)

Statistically different from control body weight gain: *= $p \leq 0.05$; **= $p \leq 0.01$

[a] net body weight change = [body weight (day 20) minus weight of gravid uterus] minus body weight (day 0)

[b] No statistical evaluation were performed for the overall time frames day 6–16 and 0–20

Maternal necropsy:
No remarkable necropsy findings were observed at any dose level. The mean net body weight change was significantly decreased in the mid- and high-dose group by 14 % and 32 % relative to control, respectively (Table B.6.6-27).
Reproductive parameters:
No treatment-related effects on fertility, mating and gestation indices were observed. An adequate number of litters was available for evaluation in all treatment and control groups.
In conclusion, no adverse maternal effects were observed in the 3 mg/kg bw/d dose group.
Results embryo/foetotoxicity:
Embryo implantation/resorption:
A minimally increased, albeit not statistically significant and within the historical control range, increase in post-implantation loss and early resorptions occurred in the 40 mg/kg bw/d dams.

Table B.6.6-28: Rat developmental toxicity: Implantation data

	Incidence of implantation data			
	0 mg/kg bw/d	3 mg/kg bw/d	10 mg/kg bw/d	40 mg/kg bw/d
Post-implanation loss (total)/ Mean %	21/ 6.9	17/ 6.7	14/ 6.2	29/ 9.8
Early resorptions (Total)/ Mean %	19/ 6.3	16/ 5.7	14/ 6.2	26/ 8.7

No further test-compound related effects on any reproductive indices or any embryological endpoints were noted.
Litter effects:
There were no statistically significant effects on litter size or the number of viable foetuses per litter. The sole test compound-related litter finding, a statistically significant decrease in foetal weight (male:

-8 %, female: -9 %, and combined: -9 % relative to control; p ≤0.01), was observed in the 40 mg/kg bw/d dose group.
Foetal external and visceral findings:
No test compound-related foetal external or visceral malformations or variations were observed in any dose group.
Foetal skeletal findings:
No statistically significant increases in the incidence of specific or total skeletal malformations were observed at any dose level.
Skeletal variations observed that were considered treatment-related are summarised in the next table. Significantly increased foetal incidences of enlarged anterior fontanel and ossification disorders of frontal bones, sacral and caudal arches, metacarpals, sternebrae segments and xiphoid were observed at the highest dose level of 40 mg/kg bw/d. Corresponding litters incidence were increased in most cases, albeit none to a statistically significant degree. Although test compound-related, these findings are considered secondary to the severe maternal toxicity (which included mortality) and the resultant retardation in foetal development, as evidenced by the statistically significantly decreased foetal weight, observed at this dose level. No effect on the foetal or litter incidence of total skeletal variations was observed.

Table B.6.6-29: Rat developmental toxicity study: Foetal skeletal findings

Incidence of foetal skeletal findings				
Finding	Dose group (mg/kg bw/d)			
	0	3	10	40
Foetuses evaluated	27	24	21	23
Litters evaluated	152	145	127	133
Frontal bones, incompletely ossified – foetal incidence (%)	57.2	50.3	57.5	72.9*
– litter incidence (%)	96.3	87.5	95.2	100
Anterior fontanel, enlarged – foetal incidence (%)	59.9	50.3	61.4	74.4*
– litter incidence (%)	100	87.5	95.2	100
Ribs, presence of ossification centres – foetal incidence (%)	27.6	29.0	28.3	14.3*
– litter incidence (%)	66.7	79.2	76.2	52.2
Sacral arches, incompletely ossified – foetal incidence (%)	58.6	55.9	60.6	88.0**
– litter incidence (%)	92.6	87.5	95.2	100
Caudal arches, unossified – foetal incidence (%)	41.4	44.1	40.2	63.9**
– litter incidence (%)	77.8	83.3	76.2	100
Metacarpals, incompletely ossified – foetal incidence (%)	26.3	18.6	29.9	39.8*
– litter incidence (%)	63.0	62.5	66.7	91.3
Sternebrae segment 2, incompletely ossified – foetal incidence (%)	13.2	11.7	18.9	25.6*
– litter incidence (%)	48.1	37.5	52.4	60.9
Sternebrae segment 5, unossified – foetal incidence (%)	10.5	4.8	16.5	27.8**
– litter incidence (%)	40.7	29.2	42.9	73.9

Incidence of foetal skeletal findings				
Finding	Dose group (mg/kg bw/d)			
	0	3	10	40
Xiphoid, unossified – foetal incidence (%)	2.0	1.4	3.9	11.3**
– litter incidence (%)	11.1	8.3	19.0	34.8

*= $p \leq 0.05$; **= $p \leq 0.01$

Conclusion:

Beta-cyfluthrin technical, administered as described in this study, produced maternal toxicity at doses of 10 and 40 mg/kg bw/d. The 3 mg/kg bw/d dose was free of test compound-related maternal effects. Developmental effects: reduced foetal weight and increased foetal skeletal variations were observed in the 40 mg/kg bw/day dose group. No other dose groups exhibited test compound-related developmental effects and no embryotoxicity was observed at any dose level.
Based on the observation of developmental effects only at a dose level that produced maternal lethality, the developmental findings are considered secondary to maternal toxicity. Therefore, beta-cyfluthrin technical is not considered a primary developmental toxicant.
NOAEL (developmental toxicity): 10 mg/kg bw/d

Re-evaluation by the RMS (2015):

40 mg/kg bw/d:
Mortality and clinical findings (hypoactivity, locomotor incoordination, salivation) in dams; decreased body weight gain and food consumption of dams; minimally increased, albeit not statistically significant and within the historical control range, increase in post-implantation loss and early resorptions; decrease in foetal weight; ossification disorders of frontal bones, sacral and caudal arches, metacarpals, sternebrae segments and xiphoid.
10 mg/kg bw/d:
decreased body weight gain and food consumption of dams.
3 mg/kg bw/d:
No adverse maternal and offspring effects.
NOAEL maternal: 3 mg/kg bw/d
NOAEL developmental: 10 mg/kg bw/d
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

Literature research for the Renewal Assessment Report (RAR):

Data point:	KIIA 5.10
Report:	Syed et al. (2010) ASB2015-933: Evaluation of teratogenic potential of cyfluthrin, a synthetic pyrethroid in Swiss albino mice. Toxicology and Industrial Health 26(2), 105-111. Soni et al. (2010) ASB2015-925. Perinatal toxicity of cyfluthrin in mice: Developmental and behavioural effects. Human and Experimental Toxicology 30(8) 1096-1105. Shafer and Crofton (2011) ASB2015-932. Comments on: 'Perinatal toxicity of cyfluthrin in mice: Developmental and behavioural effects' by Soni and colleagues.
Guideline(s):	Not applicable

Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Not acceptable

Materials and methods:

Pregnant swiss albino mice (number unknown (Syed et al., 2010, ASB2015-933) or 10 animals/group (Soni et al., 2010, ASB2015-925), source: Indian Veterinary Research Institute, Bareilly (UP), age 60 days, weighing 20-25 g) were orally administered two doses of SOLFAC 050 EW (containing 5 % cyfluthrin, batch and purity not reported; formulation ingredients not reported) at 16 mg/kg bw and 32 mg/kg bw (in 0.1 mL tap water) daily during days 5-14 (group 1) and during days 14-18 of gestation (group 2).
Dams were sacrificed on day 18 of gestation (cervical dislocation). Implantation sites, number of live and dead foetuses, implantation sites, resorption sites, placental weights, and number of corpora lutea were recorded. Foetal weights (live foetuses) and gender were determined. External, skeletal and visceral examinations were performed. Behavioural tests on neonates (pivoting, tail hang reflex, surface righting reflex) and weanlings (open field studies) were conducted in the study of Soni et al. (2010, ASB2015-925).
Statistical methods: Mann-Whitney *U*, Student's *t* test

Results and discussions:

Maternal observations: No mortality occurred. Burrowing behaviour and reduction in bw gain after administration of 32 mg/kg bw SOLFAC 050 EW.
Offspring toxicity: Reduced litter size, reduced number of live foetuses, lower foetal bw, increased number of resorbed foetuses after administration of 32 mg/kg bw SOLFAC 050 EW at days 5-14.
Increased number of dead and resorbed foetuses, lower foetal bw after administration of 32 mg/kg bw SOLFAC 050 EW at days 14-18.
Hydrocephaly, anophthalmia, microphthalmia, pulmonary edema and subcutaneous edema were reported for both doses at administration days 5-14 and days 14-18.
Some skeletal changes (rib and sternal defects, reduced ossification of fore- and hindlimb phalanges) occurred after administration days 5-14 and days 14-18, although not dose-related.
In the neonates, pivoting activity, righting reflex and the tail hang reflex were affected/delayed/reduced. The weanlings locomotion and exploration were affected at the high dose.

Conclusion:

The study is considered not acceptable based on the following serious flaws of the study design that prevent any conclusion on the developmental toxicity of cyfluthrin:
(1) The animals were administered a formulation containing 5 % cyfluthrin and 95 % other ingredients; (2) Batch number and purity of the active substance not reported; (3) The control group received tap water and not the inert ingredients of the formulation; (4) Reproductive parameters (number of implantation/resorption sites, absolute number of live and dead foetuses, litter size, placental weights, number of corpora lutea, gender of the foetuses) not reported; (5) Individual data not reported; (6) Total number of pups/foetuses not reported (live, dead); (7) Different number of foetuses for externally and internally examinations (8) The design of the behavioural studies is unclear (litter-based or pup based, number of animals not reported).

Data point:	KIIA 5.10
Report:	Zhang et al. (2008) ASB2015-918 The antiandrogenic activity of pyrethroid pesticides cyfluthrin and beta-cyfluthrin. Reproductive Toxicology 25 (2008) 491-496
Guideline(s):	Not applicable

Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract: *In vivo* and *in vitro* assays to investigate the suspected antiandrogenic activity of two pyrethroids, cyfluthrin (6, 18, or 54 mg/kg bw/d; purity 92.6 %) and beta-cyfluthrin (4, 12, or 36 mg/kg bw/d; purity 97 %) are described. A stably transfected, androgen-responsive cell line, MDA-kb2, was used to determine the androgen receptor (AR) antagonistic effects of cyfluthrin and beta-cyfluthrin *in vitro*, and the Hershberger assay was utilised to detect the antiandrogenic potential of the two pyrethroids *in vivo*. Moreover, the antiandrogenic activities of cyfluthrin and beta-cyfluthrin to four structurally related pyrethroids: permethrin, cypermethrin, beta-cypermethrin and bifenthrin were compared. The results show that cyfluthrin and beta-cyfluthrin can block 5-dihydrotestosterone (DHT)-induced AR activity in MDA-kb2 cells. In the Hershberger assay, cyfluthrin, at doses of 18 and 54 mg/kg, and beta-cyfluthrin, at a dose of 36 mg/kg, caused significant decrease in the weight of seminal vesicle, ventral prostate, dorsolateral prostate, LABC, Cowper's glands, though not significant in glans penis. Beta-cyfluthrin at dose of 12 mg/kg (vehicle peanut oil) decreased only the weight of seminal vesicle and had no effect on the other accessory sex tissues. The increase rank of antiandrogenic activity was: Beta-cypermethrin < permethrin < beta-cyfluthrin < cypermethrin < cyfluthrin < bifenthrin < flutamide.

Conclusion:

The treatment had no effect on the body weight development of the rats. Therefore, it can be considered that the organ weight decreases are not secondary to body weight decreases. The results suggest that cyfluthrin and beta-cyfluthrin might be moderate antiandrogenic chemicals and they elicit antiandrogenic effects at least partly by antagonising AR. Reproductive toxicity studies did not indicate antiandrogenic effects by cyfluthrin and beta-cyfluthrin. The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Ahmad et al. (2012) ASB2015-922 Exposure to ß-Cyfluthrin During Pregnancy Induces Teratogenicity in Murine Foetuses. Pakistan J. ZooL, vol. 44(6), pp. 1515-1519, 2012
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Not acceptable

Abstract:
Teratogenicity of a pyrethroid insecticide, beta-cyfluthrin was tested in developing foetuses of mice (15/group). For this purpose, different concentrations of insecticide i.e., 1.25, 2.50 and 5.00 µg/g body weight were prepared by dissolving it in sterilised distilled water in such a way that each 0.1 mL of the solution contains desired concentration. A single dose was given orally (not further specified) on day 6 of gestation and foetuses were recovered on day 18 of gestation (cesarian section).
The authors reported that the morphological studies of foetuses showed abnormalities including microcephaly, anophthalmia, micromelia, dysmorfogenesis, dysplasia and short tail. They further reported that morphometric studies of body weight, crown rump length, brain size, length and width of eye, length of both fore limbs and hind limbs and length of tail of foetuses showed significant ($p < 0.001$) differences against controls. From the study design, it remains unclear whether the values are added from all 15 foetuses. Calculating the ratio between crown-rump (CR) length to eye length and width, to brain, fore and hind limb size and tail length, nearly the same values result for the control and high dose foetuses. Lower body weight development and a retarded growth is well known for foetuses re-

ceiving high doses of beta-cyfluthrin. In this study it is obvious that the foetuses of the high dose group had also a retarded growth. Since no morphological and morphometric methods are described in detail, the RMS is in doubt whether the anomalies reported (microcephaly, anophthalmia and short tail) are true malformations or simply represent retarded foetal growth.

Conclusion:

The study is considered not acceptable based on the following serious flaws of the study design that prevent any conclusion on the developmental toxicity of cyfluthrin:
(1) Purity, batch number and the supplier of beta-cyfluthrin not reported; (2) Administration solution for control group not reported (water?); (3) Reproductive parameters (number of implantation/resorption sites, absolute number of live and dead foetuses, litter size, placental weights, number of corpora lutea, gender of the foetuses) not reported; (4) Individual data not reported; (5) Total number of pups/foetuses not reported (live, dead); (6) Techniques for evaluation of external and soft tissue findings and morphometric observation are not reported. It remains unclear how eye length and width, brain, fore limb, hind limb size and tail length are measured; (7) Historical control data are not provided; (8) Ratio between crown-rump (CR) length to eye length and width, to brain, fore and hind limb size and tail length, is not reported.

B.6.7 Neurotoxicity

One study with beta-cyfluthrin on developmental neurotoxicity was submitted for renewal (Sheets and Lake, 2003, ASB2007-2856). No further new data on neurotoxicity have been generated since Annex-I inclusion of cyfluthrin/beta-cyfluthrin and the publication of the addendum 1 (2002, ASB2014-9599). For the renewal process they were again evaluated.
A literature search for the Renewal Assessment Report (RAR) including publications from the last 10 years was performed by the RMS. The publications were considered as supplemental information. The results had no influence on the derivation of threshold values or on classification and labelling of beta-cyfluthrin.

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.8
Report:	[redacted], 1982, TOX9401943: Safety pharmacology study with FCR 1272 on oral administration - Report no.: R 2405 (December 01, 1982); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory
Acceptability:	Supplementary

(Dates of exp. work: November 1982, repetition of one test in February 1983).

Materials and methods:

The effects of cyfluthrin with regard to the central nervous system (CNS) were studied in male mice (Bor:CFW1, source: [redacted]) and male rats (Bor:WISW (SPFCpb), same source). They received cyfluthrin (batch no.: 816170019, purity 94.9 %) via single oral administration (stomach tube) in doses of 0-0.1-0.3-1 mg/kg bw. Cyfluthrin was formulated in Cremophor EL/water, which served also as negative control compound. The following tests were performed:
sleep test (10 mice/dose group, fasted overnight),
HBE test (10 mice/dose group, fasted overnight)*,
traction test (10 mice/dose group, fasted overnight),
catalepsy test(10 mice/dose group, fasted overnight),
catalepsy test (10 rats/dose group, fasted overnight),
anti-convulsive test (10 mice/dose group, fasted overnight),
test on orientation motility (6 mice/dose group, fasted approx. 3 h),
test on spontaneous motility (6 mice/dose group, fasted approx. 6 h),
linguomandibular reflex test and test on neuromuscular transmission (2 groups with 3 unfasted rats each; each animal in each group received all the doses in increasing strength at intervals of two hours).

*The HBE test provides information for effects on central co-ordination capability (balance rod) as well as analgesic (hot plate) and anti-convulsive (electric shock) effects.
Statistical methods: Chi-square-test.

Results and discussions:

Cyfluthrin at a dose of 1.0 mg/kg bw orally prolonged and deepened the hexobarbital narcosis in the sleep test. No further effect of pharmacological/toxicological importance was noted.

Conclusion:

The compound showed no analgesic, anti-convulsive, muscle-relaxant and cataleptic properties. It did not affect the central co-ordination ability, the linguomandular reflex and the neuromuscular transmission as well as the spontaneous and orientation motilities.

Re-evaluation by the RMS (2015):

NOAEL: 0.3 mg/kg bw
Like in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study is considered as supplemental information, due to the lack of standardisation of the tests performed.

Data point:	KCA 5.8
Report:	[redacted], 1984, TOX9401944: Study of FCR 1272 on neuromuscular dysfunction in the tilting plane test on rats - Report no.: R 2896 (May 04, 1984); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory
Acceptability:	Not acceptable

(Dates of exp. work: February to April 1984)

Objective of the study: The neurotropic effect of cyfluthrin was investigated in the so-called slip-angle test, which is designed to provide information on pharmacokinetic effects of drugs on locomotor function. This study was done to supplement findings of a safety pharmacological study [redacted], 1982, TOX9401943).

Materials and methods:

Groups of 10 male rats (Bor:WISW (SPFCpb), source: [redacted]) received cyfluthrin (batch no.: 070682, purity: not specified) via single oral administration by stomach tube. The doses were 0-0.1-0.3-1.0 mg/kg bw (1st experiment) or 0-0.01-0.03-0.1 mg/kg bw (2nd experiment). The positive control substance was diazepam (5 mg/kg bw). As reference substance cypermethrin (0.1-0.3-1 mg/kg bw) was used. Cyfluthrin and cypermethrin were formulated in Cremophor EL/water, which served also as negative control compound. Diazepam was formulated in tragacanth mucilage. Slip angle test: For each rat, the angle at which the animal started to slide down from a tilting plane was recorded five times at each time of measurement (30 min and 2, 5 and 7 h after administration).

Results and discussions:

Single oral administration of cyfluthrin at dose levels from 0.03 mg/kg bw onward impaired the gripping ability of the rats at some times of measurement. At the dose of 0.03 mg/kg bw the slip angle was significantly reduced after one time of measurement (7 h). No effect was observed at the dose of 0.01 mg/kg bw.
With the reference substance cypermethrin (also an α-cyano-pyrethroid) the slip angle was reduced at the dose of 0.3 mg/kg bw, though 10-fold higher dose, after 5 h only.

Conclusion:

The threshold dose for neuromuscular dysfunction was 0.03 mg/kg bw in this study.

Re-evaluation by the RMS (2015):

The study is considered not acceptable since no information on the purity of the test substance is given. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as supplemental information.

Data point:	KCA 5.8
Report:	[redacted], 1985, TOX9401945 CNS safety pharmacology study with BAY VL 1704 on oral administration - Report no.: R 3459 (July 19, 1985); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory
Acceptability:	Not acceptable

(Dates of exp. work: July 08 to 19, 1985).

Materials and methods:

The effects of cyfluthrin with regard to CNS were studied in male mice (Bor: CFW1, source: [redacted]) and male rats (Bor:WISW (SPFCpb), same source). They received cyfluthrin (batch no.: 233490583, purity: not specified) via single oral administration (stomach tube) in doses of 0-3-10-30 mg/kg bw. Cyfluthrin was formulated in polyethylene glycol 400, which served also as negative control compound. The following tests were performed:
sleep test (10 mice/dose group, fasted overnight),
HBE test (10 mice/dose group, fasted overnight)*,
traction test (10 mice/dose group, fasted overnight),
catalepsy test(10 mice/dose group, fasted overnight),
catalepsy test (10 rats/dose group, fasted overnight),
anti-convulsive test (10 mice/dose group, fasted overnight),
test on orientation motility (6 mice/dose group, fasted approx. 3 h),
test on spontaneous motility (6 mice/dose group, fasted approx. 6 h),
linguomandibular reflex test and test on neuromuscular transmission (5 unfasted rats, each animal received all the doses in increasing strength at intervals of one hour).

*The HBE test provides information for effects on central co-ordination capability (balance rod) as well as analgesic (hot plate) and anti-convulsive (electric shock) effects.
Statistical methods: U-test of Wilcoxon, Mann, Whitney, Chi-square-test.

Results and discussions:

In the sleep test no potentiation of anaesthesia was found at 3 and 10 mg/kg bw cyfluthrin. In this test the dose of 30 mg/kg bw caused 60 % mortality and seizures in all mice. Thus, no statement was possible on the effect of cyfluthrin regarding the sleeping time at this obviously toxic dose.
Doses up to 30 mg/kg bw were without analgesic, anti-convulsive and muscle-relaxant properties.

Conclusion:

These doses did not affect the central co-ordination capability, the orientation and spontaneous motilities as well as the linguomandibular reflex and the neuromuscular transmission. The same doses did not cause catalepsy (with the limitation, that at the dose of 30 mg/kg bw by reason of toxic side-effects (disappearance of righting reflex, the inability to hold oneself on the rod, prostration) three mice could not be evaluated).

Re-evaluation by the RMS (2015):

The study is considered not acceptable since no information on the purity of the test substance is given. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as supplemental information.

Data point:	KCA 5.7.1
Report:	[redacted], 1982, TOX9401922: FCR 1272 - Study for nerve damage effect on the rat after 5-months oral application. Report no. 10705 (10.03.1982); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(Dates of exp. work: January 1980 to June 1980).

Materials and methods:

Groups of 15 male and 15 female rats (Wistar TNO/W 74; source: [redacted]) received cyfluthrin (batch no. 16001/79; purity: 83.3%; vehicle: polyethylene glycol 400) daily by gavage for 5 months at doses of 30-80 mg/kg bw/d (30 mg/kg: 1 d, 40 mg/kg: 4/5 d, 50 mg/kg: 7 d, 60 mg/kg: 100/71 d, 80 mg/kg: 42/69 d; in males/females). A further group of 15 males and 15 females served as untreated control.
Clinical examinations: behaviour and appearance, mortality: daily; body weight: weekly; N-demethylase, O-demethylase and cytochrome P 450 (in liver tissue): at termination.
Pathology: gross necropsy: all animals which died or were sacrificed at termination; organ weights: liver, kidneys, brain (all animals sacrificed at termination); histopathology: liver, kidneys, adrenals, brain, spinal cord, sciatic nerve (5 animals/sex/group at termination).
Statistical methods: Body weight, organ weights, clinical chemistry data: means, standard deviation.

Results and discussion:

An increased mortality was found in males (control group: 2 males, 2 females; treated group: 8 males, 2 females). Acute toxic symptoms from 60 mg/kg bw/d onwards (digging and grooming movements, tremor, uncoordinated or stretched gait, salivation) for 2 to 4 h after each application were observed. The body weight gain was decreased in males of the 60 mg/kg bw/d group (body weight of control/treated group at termination: 381/283 g). Furthermore in the males the absolute liver and kidneys weights were reduced. The gross pathology revealed only non-specific findings on the liver (lobulation) and on the kidneys (mottled surface).
No abnormalities were detected in the clinicochemical and histopathological investigations.

Conclusion:

Repeated oral administration of cyfluthrin to rats at doses up to 80 mg/kg bw for 5 months showed no evidence of neurotoxic damage attributable to the test substance.

Re-evaluation by the RMS (2015):

No NOAEL could be established in this study. The study is considered supplementary. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable for screening of neurotoxic potential in rats.

Data point:	KCA 5.7.1
Report:	[redacted], 1983, TOX9401923 FCR 1272 - Special toxicological study (morphological effects on the nervous system of rats). Report no. R 3362 (30.06.1983); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was conducted, GLP was not compulsory
Acceptability:	Supplementary

(Dates of exp. work: August 1982 to June 1983).

Objective of the study: The study was done to confirm the quality and recovery of a minimal axonal degeneration in the sciatic nerve which was microscopically found in some rats from the 1000 ppm group in a 4-week feeding test ([redacted] et al., 1982).

Materials and methods:

A group of 50 male rats (Sprague-Dawley; source: [redacted].) received cyfluthrin (batch no. 81617009; purity: 95 %; vehicle: polyethylene glycol 400) daily by gavage for 14 days at a dose level of 80 mg/kg bw/d. As clinical signs of a mild to severe nature occurred, the dose was reduced to 40 mg/kg bw/d on days 6-11 and 13 of the treatment. A group of 25 male rats served as untreated control. Each 10 rats of the treated group and 5 rats of the control group were sacrificed 1 or 5 days and 1, 2 or 3 months after the final administration.
Clinical examinations: behaviour and appearance, mortality (all animals): daily; body weight (all animals): daily on the administration period, and at day 1 and 5, week 2, and month 1, 2 and 3 of the post-treatment observation period.
Pathology: histopathological examinations of the brain, spinal cord (cervical, thoracic, lumbar), sciatic and femoral nerves, femoral and gastrocnemial muscle (10 treated/5 control rats): at each sacrifice time; electron-microscopical examinations of the cerebral cortex, vermis cerebelli, spinal cord (cervical: ventral and lateral funiculus; thoracic and lumbar: ventral funiculus), sciatic and femoral nerves, femoral and gastrocnemial muscle (2 treated/1 control rats): at each sacrifice time except for 2nd month. Statistical methods: none.

Results and discussions:

Acute toxic symptoms (abnormal gait, salivation, red tears) were observed for several hours after each application. 28 out of 50 and 1 out of 40 rats had abnormal gait on day 1 and day 5 after cessation of treatment, respectively. The body weight development decreased during the treatment period. A normal weight gain was observed after cessation of treatment.
Histopathological analysis revealed minimal degree of single fibres degeneration (swelling and desquamation of axon or myelin) in 6 out of 8 rats at day 1, in 3 out of 8 rats at day 5, in 3 out of 8 rats at month 1 and in 2 out of 9 rats at month 2. No changes were seen at month 3.
Electron-microscopic examination of sciatic nerve revealed dilatation of neurotubules, proliferation of neurofilaments and degeneration of mitochondria at day 1, day 5 and month 1. No changes were seen at month 3.

Conclusion:

Repeated oral administration of cyfluthrin to rats at doses up to 80 mg/kg bw for 14 days caused abnormal gait and minimal degree of axonal degeneration in the sciatic nerve. However, both of these alterations disappeared during the 3-month post-treatment period, suggesting that the treatment-induced effects were reversible.

Re-evaluation by the RMS (2015):

No NOAEL could be established in this study. The study is now considered supplementary. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable for screening of neurotoxic potential in rats.

Data point:	KCA 5.7.1
Report:	[redacted], 1983, TOX9401924: FCR 1272 - Study for neurotoxic effect on rats after subacute oral administration. Report no. 12338 (December 27, 1983); Addendum to the report 12338A (October 08, 1985); [redacted]
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(Dates of exp. work: March 1983).

Objective of the study: The study was done to confirm the results of the aforementioned 5-month study in the rat [redacted], 1982, TOX9401922) with the aid of modified techniques of preparation (perfusion of the animals, fixation of the spinal cord in the vertebral canal).

Materials and methods:

Groups of 5 male and 5 female rats (Wistar (Bor:WISW), source: [redacted]) received cyfluthrin (batch no. 816270030; purity: 96.5 %; vehicle: polyethylene glycol 400) daily by gavage for 14 days in doses of 0, 50 (males only) and 60 mg/kg bw/d.
Clinical examinations: behaviour and appearance, mortality and body weight (all animals): daily. Pathology: gross necropsy: all animals; histopathology (all animals sacrificed at termination): brain, spinal cord, sciatic nerves and femoral muscle. Statistical methods: Body weight means.

Results and discussions:

At 60 mg/kg bw 4 out of 5 males died between day 5 and 8. At doses of 50 and 60 mg/kg bw acute toxic symptoms (unspecific disturbed behaviour, rolling, tremor, abnormal gait, salivation) were observed from day 2 onward.
The body weight development was decreased in males at both doses.
At necropsy, no specific findings were observed.
The histopathological examination revealed no treatment-related findings in sacrificed rats. Small fresh haemorrhages were observed in the brains of dead rats.
In the addendum to Report No.12338 ([redacted], 1983, TOX9401924) individual gross and histopathology findings are reported.

Conclusion:

After repeated oral administration of cyfluthrin to rats at doses up to 60 mg/kg bw/d for 14 days, the histopathological examination of the nervous system did not reveal any evidence of neurotoxic damage attributable to the test substance.

Re-evaluation by the RMS (2015):

No NOAEL could be established in this study. The animals which died during the study exhibited circumscribed haemorrhages in the brain. They were considered most likely to be the result of a terminal cardiovascular disorder with necrosis of the vascular walls rather then a neurotoxic effect caused

by the test substance.
The study is now considered supplementary. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable for screening of neurotoxic potential in rats.

Data point:	KCA 5.8
Report:	Crofton and Reiter, 1988, TOX9401952: The effects of type I and type II pyrethroids on motor activity and the acoustic startle response in the rat - Fundam. and Appl. Toxicol. 10, 624 - 634, 1988.
Guideline(s):	No (not a standard guideline study)
Deviations:	Not applicable
GLP:	No
Acceptability:	Supplementary

Materials and methods:

Two behavioural experiments (locomotor activity and acoustic startle response) were performed on 8 week old male Long Evans hooded rats that had received oral doses of 10-50 mg cyfluthrin/kg bw in 1 mL corn oil 2 h before testing.

Results and discussions:

Cyfluthrin exhibited a dose-dependent reduction in locomotor activity as well as a decreased amplitude and an increased latency to onset of the acoustic startle response.

Conclusion:

The effects of cyfluthrin in these experiments are comparable with those of other α-cyano-pyrethroids, the so called type-II pyrethroids.

Re-evaluation by the RMS (2015):

No NOAEL could be established in this study. The study is considered supplementary. Likewise, in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as supplemental because the validity of this test is not established.

B.6.7.1 Delayed neurotoxicity studies in hens (oral application)

Data point:	KCA 5.7.2
Report:	[redacted], 1981, TOX9401918: Investigative neurotoxicity studies in hens. Report no. 165 (March 9, 1981); [redacted]
Guideline(s):	The test method partly complies with the OECD Guideline for Testing Chemicals No. 418 and 419

Deviations:	Acute study: Observation period (post-treatment) was 56 days instead of 21 days; nerve tissue sampling and histopathology were not performed. Repeated dose study: Two doses (2x 5000 mg/kg) instead of daily doses for 28 days were administered, only one dose group instead of 3 dose groups were treated, no tabulated results of histopathological and gross examinations presented. Both studies: Hens were 17 months old instead of 8-12 months, biochemical examinations (NTE) were not performed, no protective agent (i.e. atropine) to prevent death due to acute cholinergic effects was given.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(dates of exp. work: February to May 1980 - acute delayed neurotoxicity; August to September 1980 - neurotoxicity of repeated doses).

Materials and methods:

1. Study (single oral gavage application): Ten laying hens (source: [redacted], [redacted], age: approx. 17 month, weight approx. 1.5 kg) received 5000 mg/kg bw cyfluthrin (batch no.: 16003/79, purity: 84.8 %) in carbowax. The positive control group was treated with 500 mg/kg bw tri-o-cresylphosphate (TOCP) (batch no.: A7A, purity: not given).
2. Study (repeated oral application): Twenty laying hens (source: [redacted], age: 17 month, weight approx. 1.4 kg) received 2 x 5000 mg/kg bw cyfluthrin (batch no.: 16005/80, purity: 89.3 %) via oral gavage separated by an interval of 7 days. The control group (four animals) received only the vehicle carbowax. The positive control group (five animals) was treated one time with 500 mg/kg bw TOCP (batch no.: A7A, purity: not given). The hens were individually housed in suspended wire mesh cages under standardised conditions. They were maintained on a diet of Purina Layena (16 % protein) and water *ad libitum*.
Recording period: 0-56 days (1. Study), 0-49 days (2. Study).
Observations: mortality and clinical signs 0.5 and 1 hour (1. Study) as also 0.5, 1 and 4 h (2. Study) following treatment and, later on, twice daily; body weight: day 0, then weekly; gross pathology: all animals which died on a weekday and all animals which survived the treatment (day 56 or day 49, respectively). Histopathological examinations were only included in the 2. Study. Statistical methods: Not performed.

Results and discussions:

1. Study: No animal receiving cyfluthrin died. The animals lost weight in the first week though this effect proved reversible in the course of the observation period. One animal showed a pale comb, probably indicative of generally poor health. No symptoms, characteristic of delayed neurotoxicity, were observed in cyfluthrin-treated hens.
Hens receiving TOCP lost weight through day 35 of the observation period. Symptoms characteristic of delayed neurotoxicity were first observed on day 12 (ataxia, inability to stand). Mortality was first observed on day 8 and 3 hens were sacrificed in a severely moribund condition. Survivors (2 hens) showed ataxia throughout the observation period.
2. Study: One animal was sacrificed in a moribund condition.
The clinical signs on two animals were: decreased activity, increased body temperature, open-mouth breathing, ataxia. In one hen these changes were reversible, in the other hen the condition declined steadily until premature sacrifice. In TOCP-treated hens ataxia was first observed on day 9 and continuing until death or sacrifice.
Gross pathology of animals sacrificed, showed egg yolk peritonitis, pale enlarged kidneys and watery intestinal contents. Animals sacrificed at termination showed pale liver, egg yolk peritonitis and intestinal parasites.
Only in one TOCP-treated hen, in some sections of the spinal cord there were histologic changes characteristic of Wallerian degeneration.
The cyfluthrin-treated hens did not show similar degenerative changes in the central or peripheral

nervous system.
The animal sacrificed, showed findings on the kidney: a marked cellular proliferation of the glomeruli and inflammatory infiltrate mixed with globular dispersed yolk material.

Conclusion:

Cyfluthrin administered as one dose or in two weekly doses of 5000 mg/kg bw did not result in acute delayed neurotoxicity.

Re-evaluation by the RMS (2015):

Hens treated with cyfluthrin did not exhibit typical symptoms of delayed neurotoxicity and histopathological evaluation did not show signs of degenerative changes in the central or peripheral nervous system.
Due to a variety of deviations from the Guidelines this study is now considered as supplemental information. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable for screening of neurotoxic potential in chicken.

Data point:	KCA 5.7.2
Report:	[redacted], 1981, TOX9401916: Neurotoxicity studies on hens. Report no. No.: 9753 (January 21, 1981); [redacted] 1985, TOX9401917, Title: FCR 1272: Neurotoxic study with chickens- Commentary on report no. 9753 of 27.1.1981
Guideline(s):	At the time the study was performed, no particular guideline method was compulsory. The test method is partly comparable to the OECD Guideline for Testing Chemicals No. 418 and 419
Deviations:	Single dose: Observation period (post-treatment) was 28-42 days instead of 21 days. Repeated dose study: Two/five doses (2/5x 5000 mg/kg) instead of daily doses for 28 days were administered, only one dose group instead of 3 dose groups were treated, observation period was 21+62/43 days instead of 14 days. All studies: Hens were 15-20 months old instead of 8-12 months, biochemical examinations (NTE) were not performed, no protective agent (i.e. atropine) to prevent death due to acute cholinergic effects was given, lack of an untreated control group.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(dates of exp. work: October 4, 1979 to May 27, 1980).

Materials and methods:

White Leghorn laying hens (source: [redacted]) were used in all three experiments. The hens were 15 to 20 months old and weighed between 1 and 2 kg. The hens were kept in an air-conditioned house provided with a run, and had access to an open pen with natural ground. They were fed poultry grain diet. They were acclimatised for at least 2 weeks prior to initiation of the study. The hens were administered orally by gavage. Groups were treated according to the following dosing schedule:
1st study: single doses of 1000, 2500 or 5000 mg/kg bw cyfluthrin (batch no.: 16001/79, purity: 85.3 %) (10 hens per dose level). An additional group consisting of 15 hens received 5000 mg/kg bw, too.
2nd study: two doses of 5000 mg/kg bw cyfluthrin (batch no.: 16003/79, purity 84.8 %) with a 3-week-interval (30 hens).
3rd study: daily doses of 5000 mg/kg bw (batch no.: 16003/80, purity 94.3 %) on five consecutive days

(10 hens).
4th study: a single dose of 375 mg tri-o-cresylphosphate/kg bw (TOCP) serving as positive control (5 hens). The vehicles were polyethylene glycol E 400 for cyfluthrin and peanut oil for TOCP.
The observation period after the treatment was 28 to 42 days after single administrations, up to 43 days after five administrations and 21 days after the first and 21 to 62 days after the second administration.
General observations: daily check for mortality and moribundity.
Clinical observations: behaviour and walking ability, daily. Body weight: weekly. Gross pathology: all animals. Histopathology: brain, spinal cord (cervical, thoracic, lumbar), sciatic nerves. In addition, following repeated dosing on 5 consecutive days: oesophagus and crop, stomach, small and large intestine, liver, heart, lungs, spleen, kidneys, ovaries, leg and breast muscle. Statistical methods: Not applicable.

Results and discussions:

1st study: At 5000 mg/kg bw 5 out of 10 hens died.
Clinical signs (behavioural disorders, excitation) were observed in 6 out of 10 hens for up to day 3 at 2500 mg/kg bw and in all hens up to day 5 or to death at 5000 mg/kg bw. Two hens showed behavioural disorders (uncoordinated leg movements and cramped gait) starting at day 14 and day 27, respectively. The body weight development was decreased from 2500 mg/kg bw onward. The gross pathological examination revealed spotty brittle livers, pale slightly mottled kidneys and dilated lungs. Histopathological analysis revealed slight degenerative changes in sciatic nerves in 2 hens at the dose of 5000 mg/kg bw.
2nd study: Five out of 15 hens died after the second treatment.
Clinical signs (behavioural disorders, excitation) were observed in all hens up to day 6. The body weight development was reduced. The gross pathological examination revealed spotty brittle livers, pale slightly mottled kidneys and dilated lungs. Fibre degeneration (distended myelin sheaths, swollen or fragmented axons, activated Schwann's cells) mainly in the n. ischiadici were seen in the majority of the adult hens dosed twice with cyfluthrin at 5000 mg/kg bw.
3rd study: Four out of 10 hens died.
Clinical signs (behavioural disorder, sedation, stiff gait) were observed in all hens up to day 8, 1 hen up to day 11, 1 hen from day 14 to day 22, 3 hens starting from days 25, 28 and 32, respectively. The body weight development was reduced. The gross pathological examination revealed mottled kidneys, brittled livers and emaciation. Histopathological analysis revealed degenerative changes (distention or granular disintegration of the medullary sheaths, swollen or fragmented axons, activated Schwann's cells) in sciatic nerves in all hens. Glia proliferation was seen in the brain.
4th study: No mortality occurred.
The typical signs of neuropathy of delayed onset were observed, starting from day 7 to 8 after administration. The body weight development was reduced. The gross pathological examination revealed no abnormalities. Histopathological analysis revealed degenerative changes in all regions of spinal cord and in sciatic nerves in all hens.

Conclusion:

Acute toxic effects were observed at doses of >2500 mg/kg bw. The histopathological lesions were almost exclusively located in the sciatic nerves. They were comparable to background lesions in aged hens. The re-occurrence of signs, especially in cachectic animals, is interpreted as re-emergence of signs that had persisted subclinically. Evaluation of the results is impaired by the lack of an untreated control group.
In a detailed assessment of the results of this study, it was concluded that there was no indication of delayed neurotoxicity. Both hens treated with a single oral dose of 5000 mg/kg bw which survived the first weeks following administration and developed symptoms much later lost weight before clinical signs became apparent and the signs exhibited were not typical for a delayed neurotoxic effect. For lack of appropriate controls, it is not possible to evaluate the histological results [redacted] 1985, TOX9401917).

Re-evaluation by the RMS (2015):

Acute toxic effects were observed at doses of >2500 mg/kg bw (1st study).
At the dose level of 5000 mg/kg bw cyfluthrin (2nd and 3rd study) caused damage mainly to the peripheral nerves (sciatic nerves). The symptoms (behavioural disorders, excitation, sedation, stiff gait) became evident 2 weeks after treatment and correlated with histopathological findings. In the spinal cord only minimal fibre degeneration was observed after cyfluthrin application.
Therefore, cyfluthrin administered orally at maximal possible doses of 5000 mg/kg bw induced delayed neurotoxic effects in the laying hen.
Due to a variety of deviations from the Guidelines this study is considered as supplemental information. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable (supplementary).

Data point:	KCA 5.7.2
Report:	[redacted], 1986, TOX9401920: [redacted] Acute delayed neurotoxicity study with FCR 1272 in the hen. Report no. R 3690 (April 11, 1986); RCC, CH-4452 Itingen, Switzerland
Guideline(s):	The test method is partly comparable to the OECD Guideline for Testing Chemicals No. 418 and 419
Deviations:	Repeated dose study: Two/five doses (2x 4300 /5x 1500 mg/kg) instead of daily doses for 28 days were administered, only one dose group instead of 3 dose groups were treated, observation period was 56 days instead of 14 days, no protective agent (i.e. atropine) to prevent death due to acute cholinergic effects was given
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(dates of exp. work: October 14, 1985 to April, 11, 1986).
The study was conducted to repeat the aforementioned experiment (Thyssen et al., 1981, TOX9401916).

Materials and methods:

White Leghorn hens (source: [redacted]; approx. 12 months old; bw: 1.2-2.0 kg) were administered cyfluthrin (batch no. 233 590 478, 93.5 % pure) in polyethylene glycol 400 via oral gavage into the crop. The first treatment group consisting of 12 hens received a single dose of 4300 mg/kg bw and was sacrificed 21 days after. The second group (16 animals) was treated in two oral doses of 4300 mg/kg bw with an interval of 3 weeks between. A third group of 10 hens was administered a daily dose of 1500 mg/kg bw on five consecutive days. The latter groups were both terminated on day 56 following the first administration. 7 hens once receiving 10 mL PEG 400/kg bw served as vehicle control. Another 7 animals were administered a single dose of 500 mg/kg bw of the positive control compound TOCP in corn oil.
For determination of NTE activity (neuropathy target esterase) in the brain and the spinal cord, additional experimental groups were treated with 4300 mg cyfluthrin/kg bw (single dose), 10 mL PEG 400/kg bw (negative control) or 1 x 500 mg/kg bw TOCP (positive control). Five hens of each of these groups were sacrificed at 24 h, 48 h, 72 h and 7 d after dosing.
The chicken were kept under standardised conditions and had free access to feed (Kliba Legehennen Starterfutter (Mehl) Nr. 565) and water.
Clinical examinations: behaviour and appearance, motor activity, mortality: daily; food consumption and body weight: weekly; NTE-determination in selected animals as described above.
Pathology: gross necropsy: all animals which died during the study or were sacrificed at termination; histopathology: brain, spinal cord, sciatic nerve, tibial nerve in selected animals (6 hens sacrificed at termination per dose group). In the "NTE-groups", no pathological investigations were carried out. Determination of Neurotoxic Esterase (NTE) activities was carried out according to Johnson (1972).

Statistical methods: Means; for NTE determination additionally standard deviation and Dunnett-test based on pooled variance or Steel test.

Results and discussions:

The essential effects of cyfluthrin recorded in all treated groups were aggressiveness, somnolence and emaciation. All test article and TOCP-treated hens displayed a reduced food consumption. One hen of group 1 (negative control), one of group 2, and each three of groups 4 and 5 died prematurely.
No clinical signs of axonal neurotoxicity, of either acute or delayed nature, were observed in these groups.
The birds in the TOCP-treated group developed the typical signs of neurotoxicity. Practically all chicken in this positive control group exhibited nervous damage in the medulla, spinal cord and tibial nerve.
Signs of minor focal axonal alteration were found in one tissue sample (sciatic nerve) from one hen treated twice with 4300 mg/kg bw and in one tissue sample (spinal cord) from one hen receiving five doses of 5 x 1500 mg cyfluthrin/kg body weight, too. However, based on their isolated occurrence, these findings are to be regarded as spontaneous events.
No inhibition of NTE activity was detected in the cyfluthrin-treated groups (Table B.6.7-1).

Table B.6.7-1: Oral neurotoxicity study in hens - mean NTE activity in brain and spinal cord (expressed in % of the control value)

Substance administered and dose level	PEG 400 (vehicle control), 10 mL/kg bw	TOCP (positive control), 1 x 500 mg/kg bw	Cyfluthrin, 1 x 4300 mg/kg bw
24 h after dosing			
brain	100	13	119
spinal cord	100	19	82
48 h after dosing			
brain	100	10	97
spinal cord	100	27	102
72 h after dosing			
brain	100	19	110
spinal cord	100	22	99
7 d after dosing			
brain	100	70	95
spinal cord	100	66	94

Conclusion:

There was no evidence of delayed neurotoxic activity of cyfluthrin on the clinical, histological or enzymological level in this study.

Re-evaluation by the RMS (2015):

Hens treated with cyfluthrin did not exhibit typical symptoms of delayed neurotoxicity and histopathological evaluation did not show signs of degenerative changes in the central or peripheral nervous system.
Due to a variety of deviations from the Guidelines this study is considered as supplemental information. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable for screening of neurotoxic potential in chicken.

Data point: KCA 5.7.2

Report: [redacted], 1985, TOX9401919:
Study for effect on the neurotoxic target enzyme (NTE) with the chicken (Gallus domesticus). Report no. 13821 (September 16, 1985); [redacted]

Guideline(s): No (not a standard guideline study)

Deviations: Not applicable

GLP: GLP was not compulsory at the time the study was performed.

Acceptability: Acceptable

(dates of exp. work: April 1985 - June 1985).

Materials and methods:

Laying hens (Lohmann LSL, source: [redacted], 15/group, age: 7-10 month, body weight: 1.25-1.70 kg) were orally treated with cyfluthrin (batch no.: 233490583, purity: 92.9 %) in dosages of 3 x 5000 mg/kg bw (a.i.) on 3 consecutive days. The positive control group received 3 x 100 mg/kg bw tri-o-cresylphosphate (TOCP, batch no. 25E 5268, purity: 99.1 %). The vehicle was polyethylene glycol E 400. The animals were acclimatised for four weeks and were kept under standardised conditions. At start of treatment, they were kept in single cages in a standard laying battery. They were given LA Eierblitz sole feed for laying hens and tap water *ad libitum*.
Recording period: 0-3 days; body weight: day 0; gross pathology and determination of NTE activity: 3 chickens from each group were sacrificed 24 h after the first and the second application. The determination of neurotoxic target enzyme (NTE)-activity followed the method of Johnson (1972).

Results and discussions:

All animals of the cyfluthrin treated groups had died after the third administration.
Clinical signs: negative and positive control group: slightly ruffled feathers, limpness, apathy, shrunken comb; cyfluthrin treated group: enhanced symptoms of ruffled feathers, limpness, shrunken comb, apathy as also dyspnoea, vocalisation.
Gross pathology:
Animals which died: distended crop, filled with fluid, distended lung, patchy liver. Animals sacrificed: no grossly apparent organ alterations in comparison to control.
For NTE-inhibition see next table.

Table B.6.7-2: Neurotoxicity oral study on hen - NTE-inhibition

Dose [mg/kg bw/d]	inhibition 24 hours after start of study (%)			inhibition 48 hours after start of study (%)		
	brain	spinal cord	peripheral nerve	brain	spinal cord	peripheral nerve
vehicle control	-	-	-	-	-	-
FCR 1272 [5000]	12.0	15.9	35.6	0.4	2.7	0
TOCP [100]	87.2	74.6	88.9	94.6	89.4	83.3

Conclusion:

No evidence of delayed neurotoxic activity was found, despite the fact that a very high dose within the lethal range was administered.

Re-evaluation by the RMS (2015):

No evidence of delayed neurotoxic activity was found, despite the fact that a very high dose within the lethal range was administered.
Under the conditions of the study and based on the information given in the report, the study is considered acceptable. Also in the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered as acceptable.

B.6.7.2 Delayed neurotoxicity studies in hens (percutaneous application)

Data point:	KCA 5.7.2
Report:	[redacted], 1982, TOX9401921: FCR 1272 - Cyfluthrin (Baythroid active ingredient) neurotoxicity study on chickens after cutaneous administration (cumulation tests). Report no. 10768 (March 29, 1982);[redacted]
Guideline(s):	No (not a standard guideline study; house method, according to Noakes and Sanderson)
Deviations:	Not applicable
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(dates of exp. work: April 1981 to May 1981, July 1981 to September 1981).

Materials and methods:

Ten laying hens (VALO-Leghorn, source: [redacted], weight: approx. 1.6 kg) were dermally (armpit) exposed to cyfluthrin (batch no.: 16003/80, purity: 91.4 %, batch no. 816170019, purity: 95.0 %). The hens received cyfluthrin as a paste, mixed with Cellulose MN 300, in the following dosing schedule:
5 x 5000 mg/kg bw for 23 hours/day, 5 days
5 x 0 (only 5 hens) or 15 x 5000 mg/kg bw for 6 hours/day, 5 days/week.
The hens were kept in an acclimatised pen. They had free access to a run on natural ground. They received Putt laying hens' whole meal and water ad libitum. The post-observation period was 42 days.
Clinical examinations: clinical signs: daily; body weight: weekly. Histopathology: brain, spinal cord, sciatic nerve (all animals).
Statistical methods: not applicable.

Results and discussions:

5 x 5000 mg/kg bw for 23 hours/day, 5 days (pilot study):
Two hens died. The following clinical signs were observed: disturbed behaviour and apathy, but no signs of delayed neurotoxicity. The skin was reddened, swollen and oedematous.
The body weight was decreased on the administration days.
The histopathological examination revealed no treatment-related findings.
5 x 0 (only 5 hens) or 15 x 5000 mg/kg bw for 6 hours/day, 5 days/week:
No mortality was observed. The following clinical signs were observed: apathy (also at 0 mg/kg bw) and disturbed behaviour, but no signs of delayed neurotoxicity. The clinical signs at skin (reddening, swollen, corrosion) were in control and treated animals observed, but clearly more pronounced in the treated animals.
A body weight retardation was estimated during treatment period. The histopathological examination revealed no treatment-related findings.

Conclusion:

No indication of delayed neurotoxicity was found after percutaneous application of cyfluthrin to hens.

Re-evaluation by the RMS (2015):

No indication of delayed neurotoxicity was found after percutaneous application of cyfluthrin to hens. Since the test design did not follow a guideline, this study is considered as supplemental information. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study is considered acceptable.

B.6.7.3 Delayed neurotoxicity studies in hens (inhalative application)

Data point:	KCA 5.7.2
Report:	[redacted], 1983, TOX9401865: FCR 1272 (Baythroid active ingredient) (c.n.: cyfluthrin) - Study for acute and subacute inhalation toxicity on chickens. Report no. 11558 (February 14, 1983); [redacted]
Guideline(s):	At the time the study was performed, no particular guideline method was compulsory. The test method is partly comparable to the OECD Guideline for Testing Chemicals No. 403 and 412
Deviations:	No clinical chemistry data were determined, only tissue of CNS and PNS were taken for histopathology
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	Supplementary

(dates of exp. work: December 1981 to March 1982).

Materials and methods:

Ten hens per group (White leghorn, source: [redacted], weight 1-2.5 kg) received cyfluthrin (batch number: 816 170 019; purity 95.0 %, formulated in ethanol/polyethylene glycol E 400, 1:1).
1st experiment in hens: one exposure via inhalation with >596 mg/m^3 air for 4 hours.
2nd experiment in hens: duration of exposure via inhalation with 0 or 614 (+/- 215 mg/m^3) cyfluthrin (analytical concentration) was three weeks (6 hours daily, 5 times per week).
3rd experiment in rats: one exposure via inhalation with 596 mg/m^3 air for 4 hours.
The hens were singly kept in air conditioned quarters and had access to an open-air run with natural floor. The post observation period was 6 weeks. Clinical examinations: clinical signs, body weight, gross pathology and histopathology: brain, spinal cord (cervical, thoracic and lumber areas); N. ischiadicus. Statistical methods: none.

Results and discussions:

Analysis of test atmosphere: About 50 % of the aerosol mass and about 95 % of the aerosol count possessed respirable characteristics (mean mass media aerodynamic diameter (MMAD) of <5 µm).
Findings acute inhalation-rat: Male rats were exposed to cyfluthrin (single exposure, 4-hour whole-body exposure) at a concentration of 596 mg/m^3 air. All animals died during exposure or within 8 hours post-exposure. Clinical symptoms: behaviour disturbances, dyspnoea, recumbent on side and stomach, uncoordinated movements, cramped gait, irritant effect on the visible eye and nose mucosa. They served for comparison with hens.
Findings acute inhalation-hen: A concentration of up to 614 mg cyfluthrin/m³ air was tolerated without mortalities (single exposure, 4-hour whole-body exposure). The symptoms were: irritant effects on the visible eye mucosa, non-specific behaviour disturbances, sedation. The symptoms lasted from the 1st

to 4th exposure day. From the 5th exposure day the animals treated with active ingredient did not differ from the control animals up to the end of the 6-week post-observation period. The treatment had no effect on body weight. Gross pathology showed no substance-related macroscopically apparent organ alternations. No treatment-induced neurotoxic damage was found in the histopathological examination.
Findings subacute inhalation-hen: Ten hens were exposed to cyfluthrin aerosol for 15 times, 6 hours daily, 5 times per week. No mortalities occurred. The symptoms were: irritant effects on the visible eye mucosa, non-specific behaviour disturbances. The symptoms lasted from the 1st to 4th exposure day. No dose-related neurotoxic damage was found in the histopathological examination.

Conclusion:

The maximum technically producible air concentration was tolerated without neurotoxic effects.

Re-evaluation by the RMS (2015):

The maximum technically producible air concentration was tolerated in hens without mortalities and without neurotoxic effects.
Since the test design did not follow a guideline, this study is considered as supplemental information. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436), the study was considered acceptable.

Studies evaluated in the addendum 1 to the monograph (2002, ASB2014-9599):

B.6.7.4 Special Study for Acute Oral Toxicity in Rats

Data point:	KCA 5.8
Report:	[redacted], 1999, TOX2001-1264: Cyfluthrin; Special Study for Acute Oral Toxicity in Rats (Slip Angle Test) Study-No. T 0068835 & T 1068953, Report no.: PH 29371, unpublished [redacted] (Experimental work: 13 – 28 September 1999)
Guideline(s):	No guidelines exist for this special type of acute study (partly OECD TG 423).
Deviations:	Not applicable
GLP:	Yes
Acceptability:	Acceptable (for the establishment of a pharmacological NOEL for acute neurotoxic effects)

(dates of exp. work: September 1999 to December, 1999).

Materials and methods:

Test material: Cyfluthrin, purity: 96.1 %, batch no: 380760276
Test animals: Wistar rats (Hsd Cpb: WU)
An inclined plane test in young adult female Wistar rats (SPF-bred; strain Hsd Cpb: WU; breeder [redacted] was conducted with to establish a pharmacological no-observed-effect level for acute neurotoxic effects. The vehicle for cyfluthrin was an aqueous Cremophor® EL suspension which is known to provide a high bioavailability. In groups 5-8 milk was used instead of water in order to check which effects from residues in food could result in comparison with the effects exerted by an aqueous formulation of cyfluthrin. The test substance was administered via gavage. The ability of female Wistar rats to maintain a stable position on the inclined plane was tested in groups of 5 or 10 animals orally treated with cyfluthrin doses ranging from 0.015 to 9 mg/kg bw. Triplicate

measurements of the slip angle were made prior to oral administration, and at predetermined times 0.5-24 hours later. Animals of groups 1-4 were used to check the temporal occurrence of clinical signs. Body weight development and gross necropsy was also recorded.

Results and discussions:

Clinical signs (amongst other things reduced motility, laboured breathing, increased salivation, uncoordinated gait, sternal recumbency, rolling over, diarrhea, vocalisation and temporary shaking) in all 5 animals tested were almost exclusively observed at 9 mg/kg bw starting from approx. 1 h after administration. The main surge of clinical signs had subsided after approx. 6 h, reduced motility remained. At 7.5 mg/kg bw, digging and preening movements of very short duration were observed in all 5 animals. Very slight reactions were observed in 3 of 5 animals at 3 mg/kg bw and in 1 of 10 animals at 2.5 mg/kg bw.
Changes in slip angle were not yet observable 1 h after administration of 9 mg/kg bw when many clinical signs had already been observed. Only 2 h after administration, the slip angle was significantly reduced at 9 mg /kg bw. This time point, 2 hours after administration, has to be regarded as the time of peak effect, which is also in agreement with the pharmacokinetic studies which indicated a t_{max} of 1.5-2 h, and with the occurrence of acute clinical signs. Changes in slip angle were no longer observed 6 hours after treatment when almost all clinical signs had subsided. Milk as a vehicle had no similar effect compared with an aqueous Cremophor® EL solution.
A dose of 7.5 mg/kg bw resulted in a marginal effect which, however, was not statistically significant. There were no changes in slip angle in animals treated with 0.015-3 mg/kg bw.
No gross pathologic changes were observed in animals sacrificed at the end of the study period.

Conclusion:

An oral single dose of 3 mg /kg bw is considered to be the NOAEL in the Slip-Angle test.

Re-evaluation by the RMS (2015):

An oral single dose of 3 mg/kg bw is considered to be the NOAEL in the Slip-Angle test.
The study is considered to be acceptable for the establishment of a pharmacological NOEL for acute neurotoxic effects. In the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

Data point:	KCA 5.8
Report:	[redacted] et al, 1997, TOX2001-1265: An acute oral neurotoxicity screening study with technical grade FCR 4545 in Fischer 344 rats. [redacted] Report-No.: 107752, Study-No.: 96-412-GO, Bayer File-No.: 8265, unpublished (Experimental work from 11 – 20 March 1996)
Guideline(s):	US-EPA-FIFRA Pesticide Assessment Guideline No. 540/09-91-123, PB 91-154617)
Deviations:	None that compromised the validity of the study results
GLP:	Yes
Acceptability:	Acceptable

(dates of exp. work: March 1996 to October, 1997)

Materials and methods:

Test material: Technical grade beta-cyfluthrin, batch-no: 3030125/0250074, purity: 96.9-97.3 %
Test animals: Fischer 344 rats
Technical grade beta-cyfluthrin was administered by gavage in a single dose to fasted male and female Fischer 344 rats (12/sex/dose) at doses of 0–0.5–2 and 10 mg/kg bw. The test substance was heated and suspended in 1 % Cremophor® EL in deionised water. The following observations and measurements were included in the study: clinical observations, mortality checks, body weight, automated measurements of activity (figure-eight maze), a functional observational battery (FOB), brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves), and tissues from the central nervous system were examined microscopically for lesions.

Results and discussions:

No deaths occurred at any dose level prior to scheduled terminal sacrifice, 15 days following administration. Compound-related clinical signs (e.g. oral stain in both sexes; urine stain in males) were evident at 10 mg/kg bw. An increased incidence of peri-anal staining was observed at 2 and 10 mg/kg bw in both sexes, but with regard to the relative high incidences of this clinical sign in control and low dose animals, it was not considered as an adverse compound-related effect.

Table B.6.7-3: Clinical observations in rats on the day of treatment

Sex	Males				Females			
Dose (mg/kg bw)	0	0.5	2	10	0	0.5	2	10
Animals examined	12	12	12	12	12	12	12	12
Oral stain	-	-	-	10	-	-	-	9
Urine stain	-	-	-	4	5	3	2	5
Peri-anal stain	8	8	11	12	5	6	11	11

The compound-related signs were apparent in both sexes on the day of treatment and resolved by day 5 following treatment.
Body weight was not affected by treatment in males or females at any dose level.
For the functional observational battery (FOB), the following compound-related and significant effects were evident on day 0 in males at 10 mg/kg bw: Gait abnormalities (incoordination), decreased activity, oral, perianal and urine stains, lying flattened, bizarre behaviour (writhing), no reaction to touch response, abnormal righting reflex, lower body temperature and prolapsed; Female animals at 10 mg/kg bw also showed gait abnormalities (incoordination), decreased activity, oral, perianal and urine stains, and an abnormal righting reflex. In addition, salivation occurred. All changes were reversible and the animals appeared normal on days 7 and 14 after treatment. A small number of the behavioural functions registered were slightly, but not significantly increased in only a few animals that received 2 mg/kg bw. Chewing movements in few animals, which were ascribed to a local effect of the test substance on the oral mucosa, were observed in few animals at all dose levels. This open field finding was confirmed in the home cage only at the highest dose level.
With regard to decreases in motor and locomotor activity, these effects were statistically significant for first two 10 min. intervals in males and the first three 10 min intervals in females, but not for the entire 90-minute test session. Additionally, a significant higher decrease was observed in female rats of the 2 mg/kg bw group only in the 3rd interval, which is not considered to be a toxicologically adverse effect. Complete recovery occurred in males and females by the next test occasion, seven days following treatment. Habituation was not affected by treatment with beta-cyfluthrin.

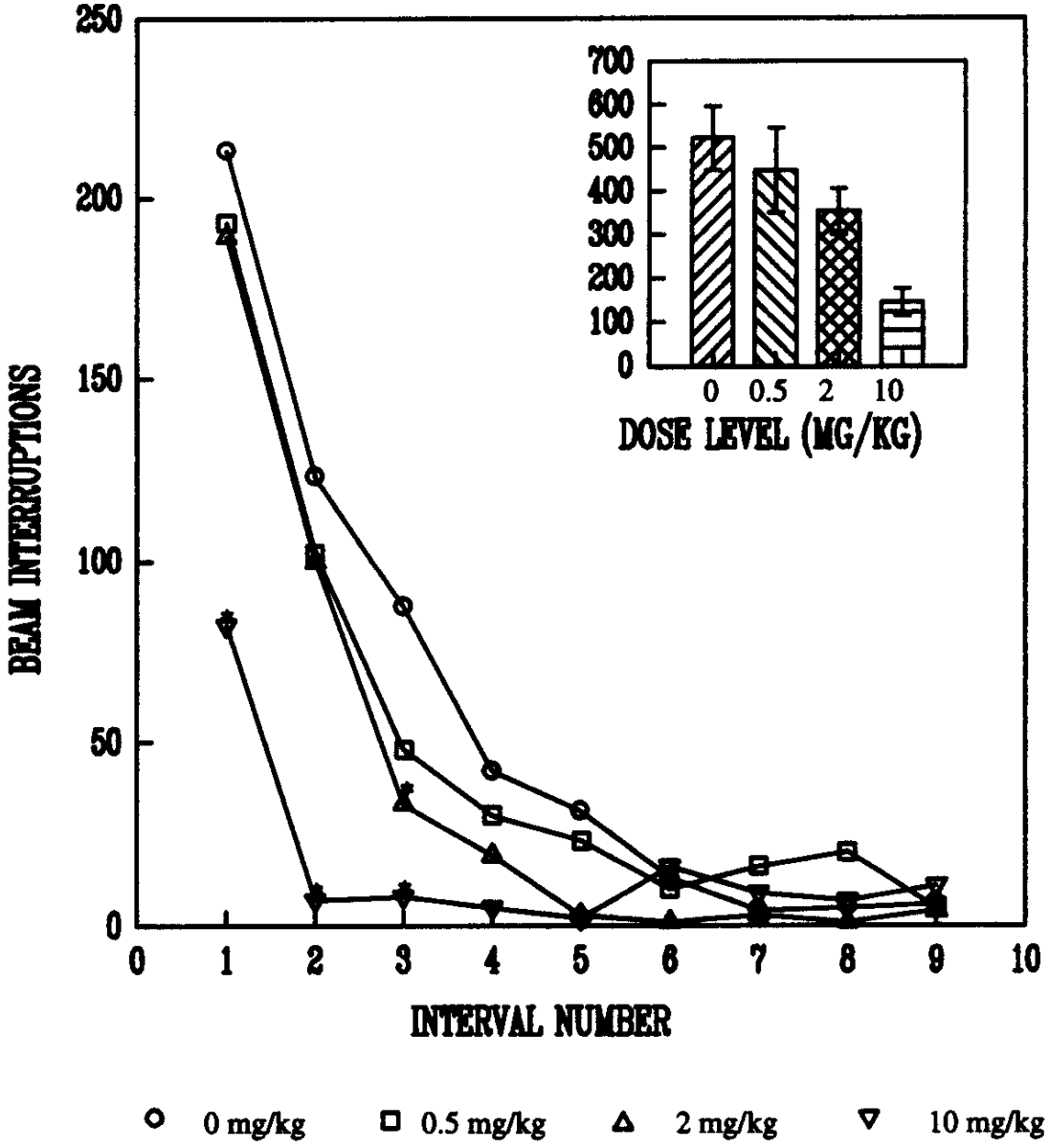


Figure B.6.7-1: Female motor activity on the day of treatment

Motor activity is expressed as the number of beam interruptions for each 10-minute interval and for the entire 90-minute test session (inset; mean + S.E.) (*p<0.05, ANOVA).
There were no compound-related gross lesions in males or females at terminal sacrifice. Brain weight was not affected by treatment in males or females at any dose level. Compound-related microscopic lesions were not evident in the high dose males or females.

Conclusion:

Based on the above mentioned findings (clinical signs, functional observational battery, motor and locomotor activity) at 10 mg/kg bw, the overall NOAEL of this acute neurotoxicity study is 2 mg/kg bw for males and females. Evidence of toxicity resolved within 7 days following treatment. It should be taken into account that the formulation with an aqueous vehicle resulted in a distinct higher acute toxicity (oral LD_{50} in rats with Cremophor EL/water: 16.2 mg/kg bw), which is to be attributed to faster and more complete enteric absorption.

Re-evaluation by the RMS (2015):

The applicant proposes a NOEL of 0.5 mg/kg bw for the FOB for males and females, based on a decreased approach response and clear oral stains in males and a decreased approach response and decreased activity in the open field in females at 2 mg/kg bw. Likewise, perianal stain observed in rats treated with 2 mg/kg bw were considered treatment-related by the applicant. Therefore, an overall NOAEL of 0.5 mg/kg bw is proposed.
The RMS considered the changes in the behavioural functions in animals at 2 mg/kg bw not as adverse effects. With regard to incidence and severity the changes were too slight and not significantly deviating from the control and low dose group. Likewise, perianal stain in animals of the 2 mg/kg bw group are considered not as an adverse compound-related effect with regard to the relative high incidences of this clinical sign in control and low dose animals (8/8/11/12 in males; 5/6/11/11 in females).
Therefore, contrary to the proposal of the applicant, a NOAEL of 2 mg/kg bw is proposed. Likewise, in the addendum 1 to the monograph (2002) a NOAEL of 2 mg/kg bw was derived.

The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

Data point:	KCA 5.7.2
Report:	[redacted], 1997, TOX2001-1266: A subchronic neurotoxicity study with technical grade FCR 4545 (β-Cyfluthrin) in Fischer 344 rats. [redacted]. Report-No.: 107491, Study-No.: 95-472-FG, Bayer File-No.: 8157, unpublished
Guideline(s):	US-EPA-FIFRA Pesticide Assessment Guideline No. 540/09-91-123, PB 91-154617
Deviations:	None that compromised the validity of the study results.
GLP:	Yes
Acceptability:	Acceptable

(dates of exp. work: September 1995 to December, 1995).

Materials and methods:

Test material: Technical beta-cyfluthrin ("BAY FCR 4545 Technical")
Batch-No. 3030125 / 0250074, purity: 96.5 % - 97.3 %)
Test animals: Fischer 344 rats
Beta-cyfluthrin was administered in the diet for 13 weeks to young-adult male and female Fischer 344 rats (12/sex/dose) at nominal concentrations of 0–30–125–400 ppm (equal to 0–2.02–7.99–26.81 mg/kg bw/d for males and 0–2.34–9.40–30.83 mg/kg bw/d for females).
All 12 rats/sex/dietary level were used for neurobehavioral evaluation, with half used for neuropathology. The following observations and measurements were included in the study: clinical observations, mortality, body weight, food consumption, automated measurements of activity (figure-eight maze), functional observational battery, ophthalmic exams, brain weight, and a gross necropsy. Skeletal muscle, peripheral nerves, eyes (with optic nerves) and tissues from the central nervous system were also examined microscopically for lesions.

Results and discussions:

There were no deaths prior to terminal sacrifice. Compound-related clinical signs were evident in males of the 125 ppm group and in males and females of the 400 ppm group. Effects in males of the 125 ppm group were limited to self-induced lesions from scratching due to paresthesias following

absorption to the skin and stimulation sensory nerve endings in the dermis. Compound-related clinical signs generally persisted with continued exposure but there was no evidence of cumulative toxicity after approximately 2 - 4 weeks of exposure.
Body weight and food consumption were reduced by treatment in males of the 400 ppm group and in females of the 125 ppm and 400 ppm groups.

For the functional observation battery (FOB), compound-related findings were apparent in both sexes at 400 ppm. They included locomotor incoordination (ataxia) and repetitive chewing movements in males and ataxia, repetitive pawing, increased reactivity, increased activity, and red nasal stain in females. These findings were transient with no evidence of cumulative toxicity after 4 weeks of exposure. The only treatment-related effects at 125 ppm are attributed to local (dermal) effects due to scratching in response to transient paresthesias caused by the pyrethroid. They included red crusty zones (scabs) about the head region, primarily the pinnae.
Automated measures for motor and locomotor activity were not affected by treatment at any dietary level. There were no compound-related ophthalmic findings.
Compound-related gross lesions were not evident in males or females at terminal sacrifice. Brain weight was not affected by treatment in either sex. There were no compound-related microscopic lesions in 400 ppm for males and females.

Conclusion:

The present feeding study with beta-cyfluthrin produced characteristic evidence of toxicity at the two highest dietary concentrations of 125 and 400 ppm. The lowest dose of 30 ppm (equal to 2.02 mg/kg bw/day) is considered to be a NOAEL in both sexes. All effects of treatment are considered reversible, with complete recovery expected with discontinuation of exposure.

Re-evaluation by the RMS (2015):

The lowest dose of 30 ppm (equal to 2.02 mg/kg bw/day) is considered to be a NOAEL in both sexes. All effects of treatment are considered reversible.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was considered acceptable.

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

Data point:	KCA 5.7.1
Report:	[redacted], 2003, ASB2007-2856 A Developmental Neurotoxicity Screening Study with Technical Grade beta-Cyfluthrin in Wistar Rats. Bayer CropScience AG Report No: 200620, M-103213-01-1 Date: 2003-07-29
Guideline(s):	U.S. EPA OPPTS Guideline 870.6300 (1998) Equivalent to OECD 426 (2007)
Deviations:	None that compromised the validity of the study results.
GLP:	Yes
Acceptability:	Acceptable

(dates of exp. work: January 2002 to April, 2002).

Materials and methods:

Test material: Technical beta-cyfluthrin ("FCR 4545 Technical"); Batch-No. 8030130 / 380566042, purity: 95.1-97.6 %).

Technical grade beta-cyfluthrin was administered via the diet from gestation day (GD) 0 through lactation day (LD) 21 to mated female Wistar rats (ca. 30 per dose level, strain: Crl:WI (Glx/BRL/Han) IGS BR rats from [redacted]) at nominal concentrations of 0, 30, 125 and 200 ppm (equal to 0, 2.4, 11.0 and 17.8 mg/kg/day, respectively during gestation and 0, 5.9, 25.4 and 40.9 mg/kg/day, respectively during lactation). The adult males served only as "breeders" and were not exposed to the test substance or included in any tests. On postnatal day (PND) 4, litters with a minimum of eight 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 (an abbreviated functional observational battery), preputial separation or vaginal patency, body weight, food consumption, body temperature, automated measures of activity (figure-eight maze), acoustic startle habituation, learning and memory (passive avoidance after weaning and a water maze task on PND 60) 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 (approx. 75 days of age) for microscopic examination and morphometry. The concentration of beta-cyfluthrin in the whole-brain from the dams (LD 21) and offspring (PND 4 and PND 21) was also measured to verify exposure. All statistical evaluations were performed using software from either INSTEM Computer Systems, SAS, or TASC. The level of significance was set at $p<0.05$, with the exception of Bartlett's test which was tested at $p<0.001$.

Results and discussions:

Findings maternal (F0 Generation):

There were no deaths prior to terminal sacrifice.

Lower body weight development during gestation day 6 was noted in high dose dams (200 ppm). During lactation (days 0-21) body weight development and food consumption was reduced in dams of the 200 ppm group.

During lactation hair loss was noted in few dams of groups 3 and 4 (125 and 200 ppm).

The FOB was unaffected in dams during gestation and lactation until PND 21.

Findings offspring:

Pup weight gain was reduced from days 11 to day 21 in pups of the 200 ppm group. Further litter data were not affected by the treatment.

Table B.6.7-4: Body weight development of pups during lactation [g ± SE]

PND	Dietary level [ppm]							
	0		30		125		200	
	Males	Females	Males	Females	Males	Females	Males	Females
0	5.8 ± 0.08	5.5 ± 0.09	5.7 ± 0.09	5.4 ± 0.08	5.8 ± 0.08	5.5 ± 0.07	5.7 ± 0.09	5.4 ± 0.10
4	9.7 ± 0.22	9.3 ± 0.24	9.2 ± 0.19	8.9 ± 0.17	9.6 ± 0.17	9.2 ± 0.18	9.0 ± 0.21	8.6 ± 0.21
11	24.7 ± 0.48	23.5 ± 0.48	23.3 ± 0.57	23.0 ± 0.55	23.9 ± 0.36	23.3 ± 0.36	22.2 ± 0.21**	21.4 ± 0.54*
17	39.0 ± 0.64	36.9 ± 0.64	37.0 ± 0.67	36.2 ± 0.65	37.3 ± 0.52	36.3 ± 0.52	35.2 ± 0.72**	34.0 ± 0.75**
21	49.6 ± 0.85	46.7 ± 0.87	46.5 ± 0.79*	45.3 ± 0.75	47.1 ± 0.65	45.6 ± 0.65	44.3 ± 0.83**	42.9 ± 0.86**

Dunnett's test*$p\leq0.05$, **$p\leq0.01$

In the FOB for pups on PND 4, minimal resistance during handling was noted for pups of the high dose group (200 ppm). No further changes were noted in animals up to PND 60.
Automated measures for motor and locomotor activity were not affected by treatment at any dietary level. There were no compound-related ophthalmic findings.
Reduced response amplitude following acoustic startle habituation was observed in male high-dose pups at PND 22. This finding was associated with reduced body weight. It was not observed at later time points, in females or other dose groups.
There were no effects of treatment on developmental landmarks (balano-preputial separation or vaginal patency).

Table B.6.7-5: Developmental landmarks

	Dietary level [ppm]			
	0	**30**	**125**	**200**
Preputial separation				
Age at landmark [days ± SE]	43.6 ± 0.34	43.9 ± 0.29	43.8 ± 0.32	44.2 ± 0.35
BW at landmark [g ± SE]	185 ± 2.0	178 ± 1.7*	178 ± 1.7*	171 ± 1.8**
Vaginal opening				
Age at landmark [days ± SE]	34.0 ± 0.27	35.0 ± 0.25*	34.4 ± 0.23	34.6 ± 0.24
BW at landmark [g ± SE]	106 ± 1.7	107 ± 1.3	105 ± 1.4	101 ± 1.1*
Pupil constriction				
Pups reaching criteria [%]	100	100	100	100

Dunnett's test, Fisher's exact test *p≤0.05, **p≤0.01

Beta-cyfluthrin was detected in brain tissue from pups on both days measured (PND 4 and PND 21) at all dietary levels, with the concentration increasing in proportion to the dietary concentration. These findings provide clear evidence of exposure during lactation.
In a 2-generation study in rats with cyfluthrin [redacted], 1996, TOX2001-1771) F_1 and F_2 pups showed coarse tremors after administration of 125 and 400 ppm (corresponding to 19 and 59 mg/kg cyfluthrin bw/d during the lactation phase). It was concluded that the presence of adverse effects in the offspring was due to transfer of cyfluthrin or of its metabolite(s) in the milk during the lactation period. In the present study no such findings were noted in the offspring.

Table B.6.7-6: Concentration of beta-cyfluthrin in whole-brain tissue

Dietary level [ppm]	**Tissue level of beta-cyfluthrin [ppm]**		
	Pups (PND 4)[1]	Pups (PND 21)	Dams (LD 21)
0	0.000	0.002	0.000
30	0.004	0.006	0.006
125	0.016	0.024	0.026
200	0.026	0.034	0.046

Based on 16-22 pups (representing a minimum 16 litters) and 18-22 dams per group.
[1] Samples were pooled to provide adequate amounts for analysis.

Compound-related gross lesions were not evident in males or females at terminal sacrifice. There were no effects on brain weight, brain morphometry or histology of brain, neural tissues or skeletal muscle at study termination.
Treatment did not affect reproduction parameters, including the fertility index.

Table B.6.7-7: Reproductive parameters

	Dietary level [ppm]			
	0	30	125	200
No. of animals cohoused	30	30	30	30
No. of animals mated	30	30	30	30
Mating index	100.0	100.0	100.0	100.0
Fertility index	86.7	96.7	96.7	86.7

Conclusion:

The overall NOAEL was 125 ppm (equivalent to 11.0 mg/kg bw/day during gestation) based on effects on body weight and food consumption in high-dose dams and effects on body weight and startle response in high-dose pups at 17.8 mg/kg bw/day.
The study is considered acceptable under the conditions of the study and based on the information given in the report.

Literature search for the Renewal Assessment Report (RAR):

Data point: KCA 5.9.1

Report: Aldridge (1990) TOX9401961
An assessment of the toxicological properties of pyrethroids and their neurotoxicity. Critical reviews in toxicology Vol. 21, Issue 2, pp. 89-103.

Guideline(s): Not applicable

Deviations: Not applicable

GLP: Not applicable

Acceptability: Supplementary

Abstract:
Most pyrethroids can be divided into two classes on the basis of differences in the signs of toxicity (T- or CSsyndromes). It is concluded that these syndromes originate from a single primary action of the pyrethroid. Pyrethroids cause morphological changes in peripheral nerves of rats when given in high doses. The morphological changes are produced as a secondary consequence of the primary action of pyrethroids and are not due to a different form of toxicity. Pyrethroids have been shown to cause-functional changes (behavioural) in rats. Increases in peripheral nerves of (3-glucuronidase and p-galactosidase have also been demonstrated. These increases are a late finding and are considered to be associated with repair processes.
Available information indicates that all the above changes are reversible or repairable. Many pyrethroids cause an effect in humans termed paraesthesia. It seems probable that parenthesis is caused by an action of pyrethroids on sodium channels of the sensory nerves.

Conclusion:

The study results are considered to represent supplemental information.

Data point: KIIA 5.7.1

Report: Wolansky et al. (2006) ASB2013-7265
Relative Potencies for Acute Effects of Pyrethroids on Motor Function in Rats. Toxicological Sciences 89(1), 271–277 (2006)

Guideline(s): Not applicable

Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
A common mode-of-action has been proposed for pyrethroids based on *in vitro* studies, which includes alterations in sodium channel dynamics in nervous system tissues, consequent disturbance of membrane polarisation, and abnormal discharge in targeted neurons. The objective of this work was to characterize individual dose-response curves for *in vivo* motor function and calculate relative potencies for eleven commonly used pyrethroids. Acute oral dose-response functions were determined in adult male Long Evans rats for five Type I (bifenthrin, S-bioallethrin, permethrin, resmethrin, tefluthrin), five Type II (beta-cyfluthrin (purity: 99.2 %; dose range: 0.05-15 mg/kg bw/d; 8 doses), lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate) and one mixed Type I/II (fenpropathrin) pyrethroids (n = 8–18 per dose; 6–11 dose levels per chemical, vehicle = corn oil, at 1 ml/kg). Motor function was measured using figure-8 mazes. Animals were tested for 1 h during the period of peak effects. All pyrethroids, regardless of structural class, produced dose-dependent decreases in motor activity. Relative potencies were calculated based on the computed ED30s (dose in mg/kg required to induce a 30 % decrease in total motor activity in figure-eight maze as compared to the corresponding vehicle-treated control group). Deltamethrin, with an ED30 of 2.51 mg/kg, was chosen as the index chemical. Relative potency ratios ranged from 0.009 (resmethrin), beta-cyfluthrin 1.136 to 2.092 (esfenvalerate).

Conclusion:

All pyrethroids produce dose-dependent decreases in motor activity. Decreases in motor activity were also noted in an acute oral neurotoxicity screening study in rats (Sheets et al., 1997, TOX2001-1265). The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Weiner et al. (2009) ASB2015-934 Comparative functional observational battery study of twelve commercial pyrethroid insecticides in male rats following acute oral exposure. NeuroToxicology 30S (2009) S1–S16
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
Twelve commercial pyrethroid insecticides (technical-grade active ingredients) were evaluated individually for acute neurobehavioral manifestations of toxicity under conditions suited to assist with determining whether they act by a common mechanism of toxicity. The pyrethroids that were tested reflect a diversity of structures, including six with an a-cyano phenoxybenzyl moiety (beta-cyfluthrin, lambda-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate and fenpropathrin) and six without this moiety (bifenthrin, S-bioallethrin, permethrin, pyrethrins, resmethrin and tefluthrin). These chemicals also present a variety of behavioural effects, including ones that are historically classified as causing a T (tremor), CS (choreoathetosis with salivation) or intermediate syndrome of intoxication, and others that have not previously been classified. Each pyrethroid that was tested consisted of the complement of isomers that occur in commercial products—a key factor for relevance for environmental and human exposure and for comparisons, since the biological activity of the individual isomers can vary tremendously. Young-adult male Sprague–Dawley rats (10 per dose group) were administered a single dose of pyrethroid by oral gavage, in corn oil, at a volume of 5 ml/kg. The control group received the vehicle alone. Each was tested at a range of two or three dose levels, including a minimally toxic dose,

to establish the more sensitive manifestations of toxicity, and a more toxic dose, to establish a more complete spectrum of neurobehavioral manifestations.
Beta-cyfluthrin (purity not reported; vehicle: corn oil) was administered at 12.5, 25, and 45 mg/kg bw. Clinical and gross observations and survival of the animals were recorded. One male of the high dose group beta-cyfluthin (45 mg/kg bw) was found dead (beside others receiving different pyrethroids) within 24 hours after treatment. Animals were evaluated using a functional observational battery (FOB) that was designed to characterise and distinguish effects classically associated with T or CS syndromes of intoxication. The FOB was performed when manifestations of toxicity were most apparent at the time of peak effect (2, 4, or 8 h post-dosing) by observers who were blinded to dose group assignment, thus avoiding possible bias. The results from this study indicate that some pyrethroids clearly exhibit the historic classification symptoms of the T and CS syndromes while others do so less obviously. Use of the statistical technique of Principal Component Analysis (PCA) further helped interpret the study findings, as described in another paper (Breckenridge et al., 2009, ASB2015-830). These results establish manifestations of neurotoxicity *in vivo* that can be used as weight of evidence to determine whether pyrethroid insecticides act through a common mechanism of toxicity in mammals. Based on a review of the FOB data, analysed by PCA, and other published data, two common mechanism groups are proposed. Group 1 would include pyrethrins, bifenthrin, resmethrin, permethrin, S-bioallethrin and tefluthrin. Group 2 would include cypermethrin, deltamethrin, esfenvalerate, beta-cyfluthrin and lamda-cyhalothrin. Fenpropathrin exhibited features of both groups.

Conclusion:

The study provides data to assist with determining whether there is evidence of one or more common mode(s) or mechanism(s) of toxicity with commercial pyrethroid insecticides as well as for possible future classification of new pyrethroids (Communication: US EPA Scientific Advisory Panel Meeting, June 16–17, 2009). Regulatory guideline acute neurotoxicity studies in rats have been conducted on some commercial pyrethroids via oral exposure but they cannot be readily compared for evidence of common effect and/or common mechanism because they used different test conditions, rat strains, vehicles, dose volumes, criteria to characterise the effect, etc. Neurobehavioral assessment of the pyrethroids is known to be particularly influenced by methodological changes in the route, vehicle, dosing volume, species and strain. Further analysis of these and other data will make a refined grouping of the pyrethroids for cumulative risk assessment.
The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Breckenridge et al. (2009) ASB2015-930 Evidence for a separate mechanism of toxicity for the Type I and the Type II pyrethroid insecticides. NeuroToxicology 30S (2009) S17–S31
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
Neurotoxicity and mechanistic data were collected for six alpha-cyano pyrethroids (beta-cyfluthrin (purity not reported), cypermethrin, deltamethrin, esfenvalerate, fenpropathrin and lamda-cyhalothrin) and up to six non-cyano containing pyrethroids (bifenthrin, S-bioallethrin [or allethrin], permethrin, pyrethrins, resmethrin [or its cis-isomer, cismethrin] and tefluthrin under standard conditions. Factor analysis and multivariate dissimilarity analysis were employed to evaluate four independent data sets comprised of (1) fifty-six behavioural and physiological parameters from an acute neurotoxicity functional observatory battery (FOB), (2) eight electrophysiological parameters from voltage clamp experiments conducted on the $Na_v1.8$ sodium channel expressed in Xenopus oocytes, (3) indices of efficacy, potency and binding calculated for calcium ion influx across neuronal membranes, membrane depolar-

isation and glutamate released from rat brain synaptosomes and (4) changes in chloride channel open state probability using a patch voltage clamp technique for membranes isolated from mouse neuroblastoma cells.
The pyrethroids segregated into Type I (T-syndrome—tremors) and Type II (CS syndrome—choreoathetosis with salivation) groups based on FOB data. Of the alpha-cyano pyrethroids, deltamethrin, lamda-cyhalothrin, cyfluthrin and cypermethrin arrayed themselves strongly in a dose-dependent manner along two factors that characterise the CS syndrome. The non-cyano containing pyrethroids were arrayed in a dose-dependent manner along two different factors that characterise the T-syndrome.

Conclusion:

The results of these analyses support the hypothesis that pyrethroids exert their toxicological effect through at least two distinct modes of action. The pyrethroids segregated themselves into the classical Type I (T-syndrome), and Type II (CS syndrome). Of the alpha-cyano pyrethroids, deltamethrin, lamda-cyhalothrin, beta-cyfluthrin and cypermethrin arrayed themselves strongly in a dose–response manner along the combined CS factor that defines the CS syndrome. The non-cyano pyrethroids (S-bioallethrin, cismethrin, resmethrin, bifenthrin and permethrin) likewise arrayed themselves in a dose-responsive manner along the combined factors that define the T-syndrome.
Although all pyrethroids induced changes in ion channel kinetics, sodium channel activation and inactivation for Type I pyrethroids was more rapid than for Type II pyrethroids. The $Na_v1.8$ sodium channel is expressed exclusively in the peripheral nervous system. It was postulated that the $Na_v1.8$ channel may mediate paraesthesia that has been reported following pyrethroid contact with skin. While it is likely that paraesthesia occurs following dermal contact to both Type I and Type II pyrethroids, it has been reported to occur more intensely, at lower doses and to last longer for Type II pyrethroids than for Type I pyrethroids.
The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Clark and Symington (2008) ASB2015-919 Neurotoxic implications of the agonistic action of CS-syndrome pyrethroids on the N-type Cav2.2 calcium channel. *Pest Manag Sci* 64:628–638 (2008)
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
Nine pyrethroids were examined on their action on ion channels at presynaptic nerve terminals, and these data were used in a cluster analysis. Type II (CS syndrome—choreoathetosis with salivation) -syndrome pyrethroids that possessed α-cyano groups (cyfluthrin and other) caused Ca2+ influx and neurotransmitter release. Type I (T-syndrome—tremors) pyrethroids did not share these actions and clustered with two non-α-cyano pyrethroids
The control of neurotransmitter release from nerve terminals involves a variety of intracellular signal mediators that converge at the α1B-subunit of the $Ca_v2.2$ channel.$Ca_v2.2$ is directly modified by deltamethrin, but the resulting perturbation is dependent upon its phosphorylation state. The findings may provide a partial explanation for the different toxic syndromes produced by structurally distinct pyrethroids.

Conclusion:

The study results are considered to represent supplemental information.

Data point:	KIIA 5.10
Report:	Guvenc et al. (2014) ASB2015-923 Evaluation of changes in monoamine levels and apoptosis induced by cyfluthrin in rats. Toxicol. Res., 2014, 3, 331
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
The aim of the study was to evaluate monoamine and mitochondrial cytochrome c levels and lipid peroxidation in adult male rats treated with cyfluthrin (14 mg kg^{-1} dose; approximately 1/10 of the LD_{50} value) for 14 days. This study also examined cyfluthrin induced apoptosis via the signalling proteins Bcl-2, caspase-9 and caspase-3, and possible anti-apoptotic effects of alpha-basic crystalline (αB-c). Levels of epinephrine, norepinephrine, and serotonin (5-hydroxytryptamine, 5-HT) in the plasma and 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) were measured in the striatum to assess neurotransmitter modification. Cyfluthrin (purity: 97.2 %, vehicle: corn oil) was administered at a daily single dose of 14 mg/kg bw/d intraperitoneally for 14 consecutive days to 16 rats. Control rats received corn oil. Twenty-four hours after the last administration the animals were killed and the brains were removed. Cyfluthrin administered to the plasma significantly reduced the levels of epinephrine and norepinephrine and increased serotonin levels, with no significant increase in lipid peroxidation. In the striatum, cyfluthrin intoxication resulted in a significant increase of the serotonin metabolite 5-HIAA with no significant increase in serotonin. Apoptosis was detected in astrocytes without a change in the level of cytochrome c but was not detected in neurons. The present study may indicate that cyfluthrin toxicity appears first in neuronal supportive cells, especially astrocytes, rather than in neurons, and that in neurons. Our findings support the hypothesis that repeated exposure to cyfluthrin alters neurotransmission of epinephrine, norepinephrine and serotonin and induces apoptosis.

Conclusion:

The study results are considered to represent supplemental information.

B.6.8 Other toxicological studies

No new studies addressing this chapter of the RAR were submitted by the applicant.
The following studies were already evaluated (DAR, 1996, ASB2010-10436 or addendum 1, 2002, ASB2014-9599). They were re-evaluated herein according to current criteria.

B.6.8.1 Toxicity studies of metabolites and relevant impurities

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

The testing facility for the following cyfluthrin metabolites (unless cis-3-[2',2'-dichloroethen-1'-yl]-2,2-dimethyl-cyclopropanecarboxylic acid and trans-3-[2',2'-dichloroethen-1'-yl]-2,2-dimethyl-cyclopropanecarboxylic acid) is Bayer AG, Institute of Toxicology, Wuppertal, Germany. When the studies were performed, GLP was not compulsory. Each evaluation is based on the today's criteria. The studies were based on (or similar to) the test guidelines listed in the respective tables.

B.6.8.1.1 3-Phenoxy-4-fluorobenzyl alcohol

The available results on 3-phenoxy-4-fluorobenzyl alcohol are summarised in the following table.

Table B.6.8-1: Summary of studies conducted with 3-phenoxy-4-fluorobenzyl alcohol

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 500-1000-1600-2000-2500 mg/kg bw female: 1000-1600-1800-2000-2240-2500 mg/kg bw (PEG 400)	male: LD_{50} = 1599 mg/kg bw female: LD_{50} = 1600-1800 mg/kg bw	- supplementary - no batch (only purity) is given	[redacted], 1987 (15419) TOX9401933
mutagenicity (Ames test) (TG 471, 1983)	bacterial strains (TA1535, TA100, TA1537, TA98)	range: 20-12500 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- supplementary - less sensitivity as only 4 instead of 5 bacterial strains were applied (E. coli WP2 or S. typhimurium TA102 not included) - 2-aminoanthracen is solely used as positive control with S9 mix	Herbold, 1987 (15909) TOX9401936

				- cytotoxic effects above 250 µg/plate - no data concerning analytic of test compound or stability (GLP requirement)	

B.6.8.1.2 3-Phenoxy-4-fluorobenzaldehyde

The available results on 3-phenoxy-4-fluorobenzaldehyde are summarised in the following table.

Table B.6.8-2: Summary of studies conducted with 3-phenoxy-4-fluorobenzaldehyde

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 100-250-500-600-750-1000-1250-1750-2500-3000 mg/kg bw female: 100-250-500-750-1000-1500-2500-3000 mg/kg bw (cremophor/water)	male: LD_{50} = 1248 mg/kg bw female: LD_{50} = 1040 mg/kg bw	- acceptable	[redacted], 1981 (9942) TOX9401927
acute dermal toxicity (TG 402, 1981)	rat (male and female)	5000 µL/kg bw (paste with cellulose)	24 h contact: LD_{50}>5000 µL/kg bw	- acceptable	[redacted], 1981 (9942) TOX9401927
acute inhalative (TG 403, 1981)	rat (male and female)	dynamic vaporization of 50 g test compound	whole body exposure for 7 h: no animal died	- supplementary - inhalable particle content and analytical concentration not given	[redacted], 1981 (9942) TOX9401927
skin irritation (TG 404, 1981)	rabbit (sex unclear)	0.5 mL test compound	negative	- acceptable - observation period only 7 days - specific data and solvent not given	[redacted], 1981 (9942) TOX9401927
eye irritation (TG 405, 1981)	rabbit (sex unclear)	100 µL	not primary irritant to mucous membranes	- acceptable - data and solvent not given - observation only for 7 days instead of 21 days	[redacted], 1981 (9942) TOX9401927

mutagenicity (Ames test) (TG 471, 1983)	bacterial strains (TA1535, TA100, TA1537, TA98)	range: 20-12500 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- supplementary - less sensitivity as only 4 instead of 5 bacterial strains were applied (E. coli WP2 or S. typhimurium TA102 not included) - cytotoxic effects above 50 µg/plate	Herbold, 1985 (13429) TOX9401928

B.6.8.1.3 3-Phenoxy-4-fluorobenzoic acid

The available results on 3-phenoxy-4-fluorobenzoic acid are summarised in the following table.

Table B. 6.8-3: Summary of studies conducted with 3-phenoxy-4-fluorobenzoic acid

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 2500, 5000 mg/kg bw female: 2500, 5000 mg/kg bw (PEG 400)	male: LD_{50}> 5000 mg/kg bw female: LD_{50}> 5000 mg/kg bw	- supplementary - no batch (only purity) is given	[redacted] 1986 (14800) TOX9401930

B.6.8.1.4 3(4'-Hydroxyphenoxy)-4-fluorobenzoic acid

The available results on 3(4'-hydroxyphenoxy)-4-fluorobenzoic acid are summarised in the following table.

Table B. 6.8-4: Summary of studies conducted with 3(4'-hydroxyphenoxy)-4-fluorobenzoic acid

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 1000 mg/kg bw female: 1000 mg/kg bw (PEG 400)	male: LD_{50}> 1000 mg/kg bw female: LD_{50}> 1000 mg/kg bw	- supplementary - no batch (only purity) is given - highest dose to low for a limit test	[redacted], 1987 (15532) TOX9401934

mutagenicity (Ames test) (TG 471, 1983)	bacterial strains (TA1535, TA100, TA1537, TA98)	range: 20-12500 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- supplementary - less sensitivity as only 4 instead of 5 bacterial strains were applied (E. coli WP2 or S. typhimurium TA102 not included) - 2-aminoanthracen is solely used as positive control with S9 mix - cytotoxic effects above 100 µg/plate - no data concerning analytic of test compound or stability (GLP requirement)	Herbold, 1987 (15724) TOX9401935

B.6.8.1.5 3-Phenoxy-4-fluorobenzoic acid amide

The available results on 3-phenoxy-4-fluorobenzoic acid amide are summarised in the following table.

Table B. 6.8-5: Summary of studies conducted with 3-phenoxy-4-fluorobenzoic acid amide

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 2500, 5000 mg/kg bw female: 2500, 5000 mg/kg bw (PEG 400)	male: LD_{50} > 5000 mg/kg bw female: LD_{50}> 5000 mg/kg bw	- supplementary - no batch (only purity) is given	[redacted], 1986 (14799) TOX9401929
mutagenicity (Ames test) (TG 471, 1983)	bacterial strains (TA1535, TA100, TA1537, TA98)	range: 20-12500 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- supplementary - batch not specified (only purity) - less sensitivity as only 4 instead of 5 bacterial strains were applied (E. coli WP2 or S. typhimurium TA102 not included) - 2-	Herbold, 1988 (16703) TOX9401938

				aminoanthracen is solely used as positive control with S9 mix - cytotoxic effects above 150 µg/plate	

B.6.8.1.6 +,-(R,S)-α-Carboxy-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2',2'-dichloroethen-1'-yl)-2,2-dimethylcyclo-propanecarboxylic acid ester (FCR 2728)

The available results on +,-(R,S)-α-carboxy-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2',2'-dichloroethen-1'-yl)-2,2-dimethylcyclo-propanecarboxylic acid ester are summarised in the following table.

Table B. 6.8-6: Summary of studies conducted with +,-(R,S)-α-carboxy-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2',2'-dichloroethen-1'-yl)-2,2-dimethylcyclo-propanecarboxylic acid ester (FCR 2728)

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 1000, 2500 mg/kg bw female: 2500 mg/kg bw (PEG 400)	male: LD_{50}> 2500 mg/kg bw female: LD_{50}> 2500 mg/kg bw	- supplementary - no batch (only purity) is given	[redacted], 1986 (15239) TOX9401931
mutagenicity (Ames test) (TG 471, 1983)	bacterial strains (TA1535, TA100, TA1537, TA98)	range: 15-12500 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- not acceptable - batch not specified (only purity) - less sensitivity as only 4 instead of 5 bacterial strains were applied (E. coli WP2 or S. typhimurium TA102 not included) - 2-aminoanthracen is solely used as positive control with S9 mix - cytotoxic effects at almost all doses	Herbold, 1988 (16687) TOX9401937

B.6.8.1.7 +,-(R,S)-α-Carboxamido-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2,2-dichloroethen-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid ester (FCR 2978, THS 3062 respectively)

The available results on +,-(R,S)-α-carboxamido-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2,2-dichloroethen-1-yl)-2,2-dimethyl-cyclopropanecarboxylic acid ester are summarised in the following table.

Table B. 6.8-7: Summary of studies conducted with +,-(R,S)-α-carboxamido-[3-phenoxy-4-fluoro]benzyl-1-(R,S)-trans-3-(2,2-dichloroethen-1-yl)- 2,2-dimethyl-cyclopropanecarboxylic acid ester

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute oral toxicity (TG 401, 1981)	rat (male and female)	male: 1000, 2500 mg/kg bw female: 2500 mg/kg bw (PEG 400)	male: LD_{50}> 2500 mg/kg bw female: LD_{50}> 2500 mg/kg bw	- supplementary - no batch (only purity) is given	[redacted] 1986 (15241) TOX9401932

B.6.8.1.8 cis-3-(2',2'-Dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid and trans-3-(2',2'-Dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid

The available results on cis-3-(2',2'-dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid and trans-3-(2',2'-Dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid are summarised in the following table. They are taken from a publication by Gaughan *et al*., 1977, TOX9401926.

Table B. 6.8-8: Summary of studies conducted with cis-3-(2',2'-dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid and trans-3-(2',2'-Dichloroethen-1'-yl)-2,2-dimethyl-cyclopropanecarboxylic acid

Endpoint	Organism (specifications)	Concentration (Vehicle)	Result	Acceptability/ Comment	Reference
acute i.p. toxicity	Swiss mice (sex unclear)	intraperitoneal administration in 20-50 µL methoxytriglycol	Cis: 48 h mouse LD_{50} = 370 mg/kg bw Trans: 48 h mouse LD_{50} = 210 mg/kg bw	- not acceptable - no purity and batch given - lack of further information	[redacted], 1977 (M-075728-01-1) TOX9401926

B.6.8.1.9 FCR 1272-Phenoxyethylester

The studies for the cyfluthrin metabolite FCR 1272-phenoxyethylester listed below were not submitted by the applicant for renewal of approval. However, the studies are available to RMS (*e.g.* from other applications).
The mean features of the studies – if evaluated as acceptable or supplementary – are presented in the following table. The evaluation is based on the today's criteria.

Table B. 6.8-9: Summary of studies conducted with FCR 1272-phenoxyethylester

Endpoint (OECD TG)	Organism (specifications)	Concentration (Vehicle)	Result	Comment	Reference
Ames test (TG 471, 1997)	bacterial strains: TA1513, TA100, TA1537, TA98, TA102	16-50-158-500-1581-5000 µg/plate; also negative control (DMSO)	negative +/- S9 mix	- 2-aminoanthracen is solely used as positive control with S9 mix - cytotoxic effects above 5000 µg/plate - substance precipitation at 5000 µg/plate	Herbold, 2002 (PH-32175) TOX2002-1391
acute oral toxicity (TG 423, 1996)	rat (male and female)	male: 2000 mg/kg bw female: 2000 mg/kg bw (PEG 400)	LD_{50}> 2500 mg/kg bw		[redacted], 2002 (PH-32041) TOX2002-1390

B.6.8.2 Supplementary studies on the active substance

B.6.8.2.1 Biochemical studies (Cyfluthrin)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point: KCA 5.8.1 /01

Report: Eben et al. 1982, TOX9401939
Comparative study of inhibition of the Na^+-, K^+- and Mg^{++}-dependent ATPase from rats and chickens brains *in vitro* by FCR 1272, some of its metabolites and further substances such as DDT, ouabain, some pyrethroids and phosphoric acid esters.
Report no.: 11116 (August 27, 1982); Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany

Guideline(s): This is not a standard guideline study.

Deviations: None.

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered acceptable.

(Dates of exp. work: not specified in the report).

The study provides additional information on the active ingredient.

Materials and methods:

The inhibition of ATPase (Na^+-, K^+- and Mg^{++}-dependent) from rats and chickens brain by cyfluthrin (batch no.: 816070017; isomer ratio: I 24.9 %, II 17.9 %, III 30.0 %, IV 22.2 %; purity: 95 %) and some of its metabolites (FCR 1476, FCR 1271, FCR 3159, FCR 1260, FCR 1261, FCR 3137, FCR 2899, FCR 3145) was measured *in vitro*. For comparison some other compounds (DDT, decamethrin, permethric acid, trichlorphon, dichlorphos, paraoxon, mipafox, NAK 1467, NAK 1654) and a specific inhibitor for the Na^+-, K^+-ATPase, ouabain, were included in this study.

Results and discussions:

Concentrations from 5 x 10^{-5} to 5 x 10^{-3} mol/L cyfluthrin induced a non-specific, non-concentration-dependent inhibition of both types of ATPases (Na^+-, K^+-ATPases: ~ 20-30 % inhibition; Mg^{++}-ATPases: ~ 10-30 % inhibition). By contrast, the enzymes were concentration-dependently inhibited by some of the metabolites (the cyanhydrins of the phenoxybenzylalcohol FCR 1271 and FCR 3159, the phenoxybenzaldehydes FCR 3137 and FCR 1260, and the phenoxybenzylalcohol FCR 1261).

Conclusion:

The IC_{50} values of both cyfluthrin and the metabolites were in the region of 10^{-4} mol/L, as compared with corresponding values of 10^{-6} mol/L and 10^{-5}mol/L for ouabain and DDT, respectively.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the results section was revised. Cyfluthrin and the other pyrethroids (but not DDT) did not inhibit the ATPase in a concentration-correlated manner (for pyrethroids: $5 \cdot 10^{-5}$-$5 \cdot 10^{-3}$ M led to 20-30 % inhibition of Na^+, K^+-dependent ATPase; 30-40 % inhibition of Mg^{2+}-dependent APTase from $5 \cdot 10^{-4}$ M). However, both ATPases were inhibited in a concentration-related manner by FCR 1271, FCR 3159, FCR 3137, FCR 1260 and FCR 1261. The IC_{50} values for Na^+, K^+-dependent ATPase were between $1 \cdot 10^{-4}$-$3 \cdot 10^{-4}$ M and for the Mg^{2+}-ATPase between $3 \cdot 10^{-4}$ and $6 \cdot 10^{-4}$ M.

Data point:	KCA 5.6.2 /09
Report:	[redacted] 1992, TOX9401940 FCR 1272 - pilot study for acid-base status following inhalation exposure to rats. Report no.: T4041207/T1041006 (November 24, 1992); [redacted]
Guideline(s):	The test was conducted only in analogy to OECD Guidelines for testing of chemicals OECD no. 403 (1981) and to Directive 92/69 EEC method B2.
Deviations:	Blood gas analysis after cyfluthrin exposure was only performed on 3 (2 male and 1 female) rats.
GLP:	The study was designated as a methodological validation study and was therefore not performed as a GLP study.
Acceptability:	The study is considered acceptable.

(Dates of exp. work: March, 16 to May 10, 1992).

The study provides additional information on the active ingredient.
Objective of the study: Inhalation toxicity studies with the rat have shown that cyfluthrin induces transient respiratory changes in this species. These changes result from sensory irritation and are manifested by reflex bradypnoea, which coincides with a reflexively induced hypothermia and respiratory alkalosis. In inhalative teratogenicity studies, the foetal development was influenced above the sensory irritant threshold concentration. No effects on the embryonic development were seen following oral administration of considerably higher doses. This pilot study was done in order to corroborate the hypothesis that a mechanistic relationship between changes in the physiological acid-base status and the influenced embryonic development exist.

Materials and methods:

Experiment 1 (study no.: T1041006): A blood gas analysis was done after CO_2 exposure and retroorbital blood sampling. Groups of 4 male Wistar rats (Bor:WISW [SPFCpb], source: [redacted], body weight: approx. 250 g, age: about 3 months) were acclimatised for two days

and then dosed at the following dosing schedule:
Control group: 10 L air/min, CO_2-group 1: 10 L air/min and over intervals of 30 min followed by 0.1-0.2-0.4-1.0 L CO_2/min (nominal concentrations), CO_2-group 2: 4 h with 10 L air/min + 0.4 L CO_2/min (nominal concentration).
Investigations: general observation: before and after the administration; body weight: before and after the administration (only group 2); rectal temperature: before and after the administration; lung function test: during the administration; blood sampling, blood gas analysis, pH and hemoglobin concentration: before and after administration (group 1 at once, group 2 30 minutes after the exposure).
Experiment 2 (study no.: T4041207): An inhalative cyfluthrin-exposure study was done with intraarterial blood sampling during the exposure. For this study 21 Sprague Dawley rats (SD, source not given, body weight approx. 250 g) were prepared with the intraarterial catheter in the Laboratory of Pharmacology and Toxicology, Hamburg and then transported by courier to Wuppertal for the further experiments. Due to technical difficulties blood gas analysis was only performed on 3 (2 male and one female) of the 21 rats. The animals were acclimatised 1 day and then received cyfluthrin (batch no. 238005176, purity 96.2 %) at a dose of 13.2 mg/m^3 (head-nose only, nebulized) air for 4 h. A mixture of PEG 400 and ethanol served as vehicle.
Investigations: rectal temperature: before and after the administration; blood sampling, blood gas analysis, pH and hemoglobin concentration: before and during the exposure (approx. 30, 60, 120, 180 and 240 min after the beginning).

Results and discussions:

Experiment 1: No clinical signs were seen. The rectal temperature was slightly lowered after the treatment. The lung function test revealed a concentration-dependent increase in minute volume. Only in the group 1-animals the blood gas analysis revealed a slight respiratory acidosis, hypercapnia (increased blood-CO_2) and a reduction in the venous oxygen partial pressure. In the group 2-animals blood gas analysis did not reveal any effect. The hemoglobin values were slightly lowered.
Experiment 2: During the exposure with cyfluthrin, the following time-dependent changes were recognised: lowering of rectal temperature, decrease in hemoglobin concentration (presumably due to repeated blood sampling), reduction in CO_2 partial pressure and increase in pH value.

Conclusion:

In experiment 1 (group 2), the induced reflectory blood gas changes normalised directly after the end of exposure. Around 30 minutes after the end of exposure no toxicologically significant changes were seen. Therefore, the only practicable way to measure the blood gas changes seems to be the measurement through intraarterial blood sampling parallel to exposure. The results of these examinations support the hypothesis that reflex bradypnoea induces secondary hypothermia. In the literature it is pointed out, that hypothermia in gravid rodents influences the development of the embryo. In connection with this the results of this pilot study corroborate the hypothesis, that exposing of rats to a greater than the sensory irritant threshold concentration (approx. 0.5 mg cyfluthrin/m^3 air in an embryotoxicity study) can induce compensatory mechanisms in thermoregulation which are tolerated by the dams, but not by the foetuses.
A distinct hypothermia developed during the 4 h exposure period (experiment 2). The determinations of the blood gases resulted in a decrease in arterial partial pressure of carbon dioxide and a rise in arterial blood pH. These results could corroborate the hypothesis that the reflex bradypnoea, which in turn has been induced by sensory irritation, induces secondary hypothermia and respiratory alkalosis. These effects are thought to influence the embryonic development.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section was revised.
Exposure of rats to concentrations of cyfluthrin above the sensory irritant threshold (~0.5 mg cyfluthrin per m^3 air) leads to reflectory respiratory changes. These changes are associated with transient effects on thermoregulation as well as the physiological acid-base status.

Further studies concerning blood gas analysis, impact on acid-base status or body temperature available to RMS

The following studies by [redacted], (1989; 16763, TOX9401870 and 1988; 17209, Z14816) and [redacted] (1991, 19852, TOX9401863) listed below were not submitted by the applicant for renewal of approval. However, the studies are available to RMS (*e.g.* from other applications).

[redacted] 1989, TOX9401870:
Cyfluthrin aerosol (analytical concentration: 16-101 mg/m³ air, 3-4 h exposure) did not induce toxicologically relevant changes in the arterial blood gases of rats. The base equivalents were slightly increased, but the pH value did not change. An increase in the arteriovenous shunt was not indicated. The rectal temperature was affected (concentration-related hypothermia beginning at 16 mg FCR 1272/m³ air).

[redacted] 1988, Z14816:
Cyfluthrin aerosol (analytical concentration: 0.3-25.1 mg/m³ air, 6 h exposure) led to hypothermia in male rats (at 3.6 mg FCR 1272/m³ air) and female rats (at 25.1 mg FCR 1272/m³ air).

[redacted], 1991, TOX9401863: The influence of cyfluthrin on body temperature was tested in male rats. The test compound was dissolved in PEG 400 and animals were treated orally with different doses (0, 125, 250 and 500 mg/kg bw). One animal died at the highest dose applied. Clinical signs were observed at all doses (*e.g.* apathy, staggering gait, labored breathing). However, the test compound had no impact on body temperature.

B.6.8.2.2 Antidote studies (Cyfluthrin)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.1 /15
Report:	[redacted] 1983, TOX9401941 Tests to determine antidote effect against FCR 1272 toxicity in rats. Report no.: 11854 (June 1, 1983); B[redacted]
Guideline(s):	This is not a standard guideline study. The LD_{50} determination was similar to OECD no. 401 (1981).
Deviations:	Concerning the LD_{50} study (cyfluthrin alone): Only one sex instead of both sexes per substance (demanded in OECD TG 1981). No necropsy performed (deviation from recommendation in OECD TG 1981 and 1987). Individual body weights and body weight development, duration of individual signs (to assess delayed effects) and individual signs per dose were not reported (recommended in OECD TG 1981 and 1987).
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(Dates of exp. work: October 1982 to December 1982).

The study provides additional information on the active ingredient.

Materials and methods:

Groups of 5 to 20 male rats (Bor:WISW [SPFCpb], source: [redacted]) received cyfluthrin (batch no.: 816170019, 816270030, purity: 94.9 %, 94.7 %) via single oral administration (for LD_{50}-determination: 10-25 mg/kg bw; for determination of antidote-effects: 10-50 mg/kg bw). When symptoms appeared, the respective antidote was administered in the following doses and application modus:
Aspisol®: 5 mg/kg bw (i.v.); Calceno "D": 10 mg/kg bw (i.v.); Methyldopa 250 Stada®: 11 mg/kg bw (i.v.); methylene blue: 10 mg/kg bw (i.v.); Myoscain®: 3.7 mg/kg bw (i.v.); sodium thiosulfate-5-hydrate: 10 mg/kg bw (i.v.); Niconacid®: 11 mg/kg bw (i.v.); Pancuronium "Organon": 0.05 mg/kg bw (i.v.); Rhex Hobein®: 86 mg/kg bw (i.v.); Thionin: 5 mg/kg bw (i.v.); acetylsalicylic acid: 5, 10 mg/kg bw (i.p.); Ergenyl®: 2.5, 25 mg/kg bw (i.p.); Musaril®: 50-300 mg/kg bw (oral), 50-400 mg/kg bw (i.p.).
The dose levels of the antidotes were based on the mean rat body weight of 200 g (converted from the manufacturer's recommended daily dose for humans).
Recording period: 0-14 days.
Statistical methods: The mean lethal dose (LD_{50}) was determined by the method of Litchfield and Wilcoxon (1949).

Results and discussions:

Table B. 6.8-10: Oral LD_{50} in fasted rats

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
Fasted male rats	cremophor/water	<10	19.6 (17.7-21.7)

= maximum dosage without clinical signs.
* = () most likely confidence interval (95 %), not specified in the report.

Onset of death: 2-3 h after exposure to FCR 1272.
Clinical signs after cyfluthrin exposure: writhing, splayed gait, uncoordinated movements, increased activity, vocalisation, salivation, difficult breathing and lethargy (clinical signs observed at all doses; 30-60 minutes after administration for up to 5 days; onset of death: 2-3 h after administration).
In these experiments substances with anti-inflammatory, analgesic, anti-epileptic, sedative or neuromuscular-regulatory activity proved insufficient as antidotes to oral intoxication with cyfluthrin. Drugs with regulatory effects on the blood pressure or circulation as well as typical cyanide antidotes and calcium also failed to antagonise the acute effects of cyfluthrin.
Intraperitoneal administration of Musaril (100 mg/kg bw) succeeded in moderate increasing the LD_{50}. Musaril also proved able to suppress the toxic signs (vocalisation, rolling = choreoathetosis) and delayed the onset of death.

Conclusion:

The administration of Musaril, a centrally-acting muscle relaxant, led to the reduced acute toxicity of cyfluthrin.

Re-evaluation by the RMS (2015):

The study is considered acceptable. It should be noted that the results section was revised.
This acute oral toxicity study with cyfluthrin (similar to OECD-Guideline no. 401 [adopted May 12, 1981]) also investigated antidote effects against cyfluthrin. It was conducted with fasted male rats. There are some minor deviations from the guideline: Only one sex instead of both sexes per substance was used. No necropsy was performed. Individual body weights and body weight development, duration of individual signs (to assess delayed effects) and individual signs per dose were not reported.
Under the conditions of the study and based on the information given in the report, the LD_{50} value for cyfluthrin (in cremophor/water) is 19.6 mg/kg bw. Clinical signs were observed at all doses. To the

symptoms observed belonged for example writhing, splayed gait and uncoordinated movements.
Under the conditions of the antidote testing study and based on the information given in the report, reduced acute toxicity was only observed after administration of Musaril®.

Data point:	KCA 5.9.6 /01
Report:	[redacted] 1984, TOX9401942 FCR 1272 - antidotal test Report no.: 271 (February 23, 1984); [redacted]
Guideline(s):	This is not a standard guideline study. The LD_{50} determination was similar to OECD no. 401 (1981).
Deviations:	Concerning the LD_{50} study (cyfluthrin alone): The purity of FCR 1272 was not given (demanded in OECD TG 1981 and 1987). Only one sex instead of both sexes per substance (demanded in OECD TG 1981). No necropsy performed (deviation from recommendation in OECD TG 1981 and 1987). Individual body weights and body weight development were not reported (recommended in OECD TG 1981 and 1987). The recording period was only 7 instead of 14 days (deviation from recommendation in OECD TG 1981 and 1987). It remains unclear whether animals were fasted.
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is not acceptable.

(Dates of exp. work: November 1983 to February 1994).

The study provides additional information on the active ingredient.

Materials and methods:

Groups of 10 male mice and rats (ICR mice, Sprague-Dawley rats, source: [redacted] received cyfluthrin (batch no.: 8241/SL/NO4, purity: not specified, vehicle: water) via single oral administration. The doses were 350-2000 mg/kg bw for mice and 1000-5600 mg/kg bw for rats.
Two antidotes were intraperitoneally injected in the following dosing schedule:
atropine sulphate (mice): 2 x 50 mg/kg bw (at 20 min and 2 h p.a. of cyfluthrin)
atropine sulphate (rats): 3 x 25 mg/kg bw (at 30 min, 3 and 24 h, p.a. of cyfluthrin)
methocarbamol (mice): 2 x 100 mg/kg bw (at 20 min and 2 h p.a. of cyfluthrin)
methocarbamol (rats): 3 x 50 mg/kg bw (1, 3 and 24 h p.a. of cyfluthrin)
atropine + methocarbamol (mice): 2 x 50 mg/kg bw + 2 x 100 mg/kg bw (same time as above)
atropine + methocarbamol (rats): 3 x 25 mg/kg bw + 3 x 50 mg/kg bw (same time as above)
Recording period: 0-7 days.
Statistical methods: The mean lethal dose (LD_{50}) was determined by the method of Bliss.

Results and discussions:

Table B. 6.8-11: Effects of antidotes on the LD_{50}

	Antidote	NOAEL [mg/kg bw][#]	LD_{50} [mg/kg bw][*]
Male mice	-	<350	660 (560-770)
Male mice	atropine	<500	840 (650-1070)
Male mice	methocarbamol	<500	970 (810-1260)
Male mice	combined	<700	1280 (1090-1510)
Male rats	-	<1000	2100 (1900-2300)
Male rats	atropine	<1400	2600 (2100-3200)
Male rats	methocarbamol	<1400	2800 (2400-3300)
Male rats	combined	<1400	3100 (2600-3700)

= maximum dosage without clinical signs.
* = () confidence interval (95 %).

Onset of death after cyfluthrin exposure in rats/mice: 1-24 h:
Clinical signs after cyfluthrin exposure - mice: salivation and titubation (after 5-10 min), athetosis and dyspnoea (after ~30 min). The symptoms diminished in the next day.
Clinical signs after cyfluthrin exposure - rats: like in mice. Salivation occurred at 10 min, titubation and athetosis from 1 h and persisted by the next day. Sporadically bradypnea and/or dyspnoea: appeared 2 h to day 2.
Each treatment of antidotes led to some degree to an antidotal effect. The greatest effect was observed after combined exposure to both antidotes.

Conclusion:

Atropine sulphate and methocarbomol (in the form of Robaxin®) exhibited only a moderate antidotal effect. The treatment with the antidote combination showed a higher protective activity.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be acceptable. However, due to deviations from the current test guideline listed below the study is now considered to be no longer acceptable. It should be noted that the results section was revised.
This acute oral toxicity study with cyfluthrin (similar to OECD-Guideline no. 401 [adopted May 12, 1981]) also investigated antidote effects against cyfluthrin. It was conducted with male rats and mice. The main reason for the decision against acceptability is the absence on information concerning the purity of FCR 1272. Furthermore, there are some deviations from the guideline in the LD_{50} study: Only one sex instead of both sexes per substance was used. No necropsy was performed. The recording period was only 7 instead of 14 days and it remains unclear whether animals were fasted. Individual body weights and body weight development were not reported.
Under the conditions of the study and based on the information given in the report, the LD_{50} value for cyfluthrin (in water) is 660 and 2100 mg/kg bw in male mice and rats, respectively. Clinical signs were observed at all doses. To the symptoms observed belonged for example salivation and athetosis.
A reduced acute toxicity was observed after administration of each antidote – and in particular after combined exposure to these antidotes.

B.6.8.2.3 Acute oral combination toxicity studies (Cyfluthrin)

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.8 /15
Report:	[redacted], 1982, TOX9401946 FCR 1272 and SIR 8514 - study for acute combination toxicity - Report no.: 10516 (January 15, 1982); [redacted]
Guideline(s):	The test is similar to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Only one sex instead of both sexes per substance (demanded in OECD TG 1981). Limit test with FCR 8514 only with one sex (deviation from recommendation in OECD TG 1981 and 1987). No necropsy was performed (deviation from recommendation in OECD TG 1981 and 1987). Purities are not given (demanded in OECD TG 1981 and 1987)*. Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG 1981 and 1987). Individual body weights and body weight development and duration of individual signs (to assess delayed effects) were not reported (recommended in OECD TG 1981 and 1987).
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered acceptable for FCR 1272 (not for SIR 8514).

(Dates of exp. work: May 1981 to September 1981)

* The purity for cyfluthrin is available from another study ([redacted], 1983, Report no.: 12003, TOX9401947)

Materials and methods:

The aim of the study was to clarify whether additive or super-additive acute toxic affects occur after single or combined oral administration of cyfluthrin (FCR 1272) and triflumuron (SIR 8514). For this purpose, cyfluthrin (batch no.: 816170019) and triflumuron (batch no.: 16001/80) were either given alone or simultaneously to unfed rats (Wistar albino rats WISW [SPF-CBP], source: [redacted], 160-180 g). Male rats were used for cyfluthrin or the combination study and female rats were used for triflumuron.
A mixture of Cremophor EL and water served as vehicle. The application volume amounted to 1 mL/100 g bw.
Dosing schedule: 0.1-0.5-10-11-12.5-15-17.5-20.5-25 mg/kg bw cyfluthrin (10 rats/dosage group), 5000 mg/kg bw triflumuron (10 rats), 500-1000-2500-5000 mg/kg bw 99.72 % triflumuron and 0.28 % cyfluthrin (10 rats per dosage group). The composition of cyfluthrin and triflumuron in the mixture was based on their percentage ratios of their LD_{50} values (equitoxic doses).
The fasted animals were fed 2 h after single dosing.
Recording period: 0-14 days.
Bw: Only at the beginning of the study.
Clinical signs were examined, necropsy was not performed.
Statistics: Calculation of mean LD_{50} values according to the method of Litchfield and Wilcoxon (1949). The theoretical LD_{50} value for the combination study was determined as published by Finney (1971) by means of the LD_{50} values for each substance and based on the assumption of an additive effect.

Results and discussions:

Table B. 6.8-12: Oral LD_{50} after 14 days in fasted rats after administration of cyfluthrin and/or triflumuron

	Substance	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
male rats	cyfluthrin	0.1	14.3 (12.8-15.9)
female rats	triflumuron	<5000	>5000
male rats	cyfluthrin + triflumuron	<500	>5000 (observed) 2526 (expected)

= maximum dosage without clinical signs.
* = () most likely confidence interval (95 %), not specified in the report.

Onset of death: between 1 and 3 h (cyfluthrin). Clinical signs after administration of cyfluthrin were observed from a dose of 0.5 mg/kg bw onward. To the symptoms belonged impaired respiration and motility, uncoordinated movements, staggering, stretched and spastic gait, rolling movements, apathy, bristling fur and at times vocalisation.
Clinial signs occurring after administration of 5000 mg/kg bw triflumuron were reduced well-being and motility as well as spastic gait.
Disturbed behaviour, accelerated respiration, reduced motility, uncoordinated movements, stretched and spastic gait, staggering and salivation were observed after administration of the mixture. There was no dose associated without clinical signs.
The factor 0.5 between the calculated and the observed LD_{50} values indicate rather a sub-additive than an additive or super-additive effect of the two substances.

Conclusion:

The study is acceptable. It provides additional information on the active ingredient.
No super-additive, but a sub-additive effect was observed after acute oral administration of equitoxic doses of cyfluthrin together with triflumuron was present.

Re-evaluation by the RMS (2015):

The study regarding FCR 1272 is still considered acceptable. It should be noted that the materials and methods section as well as the results section were revised.
This acute oral toxicity study met the basic criteria of OECD-Guideline no. 401 (adopted May 12, 1981).
The purities of cyfluthrin and triflumuron are not given. However, the purity of cyfluthrin is available from another study (report no. 12003). Therefore, the study is only considered acceptable for the experiments with cyfluthrin.
Furthermore, only one sex instead of both sexes per substance was used. The limit test with triflumuron was performed with only one sex. Food was withheld for 2 h instead of 3-4 h after substance administration. No necropsy was performed. Individual body weights and body weight development as well as duration of individual signs (to assess delayed effects) were not reported.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and triflumuron in cremophor EL/water were 14.3 mg/kg bw (male rats) and >5000 mg/kg bw (female rats), respectively. The observed LD_{50} value in the mixture was >5000 mg/kg bw (male rats). From a concentration of 0.5 mg/kg bw cyfluthrin clinical signs occurred. To the signs observed belonged for example impaired respiration and motility as well as uncoordinated movements. Oral administration of triflumuron also led to clinical signs like reduced well-being and motility as well as spastic gait. Toxic symptoms after administration of the mixture occurred at all doses. The symptoms consisted for example of disturbed behaviour, accelerated respiration and reduced motility.

Data point:	KCA 5.8 /16
Report:	[redacted] 1983, TOX9401947 FCR 1272 and SRA 5172 - study for combination toxicity - Report no.: 12003 (August 17, [redacted]
Guideline(s):	The test is similar to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Only one sex instead of both sexes per substance (demanded in OECD TG 1981). Individual body weights and body weight development or individual signs with durations were not reported (recommended in OECD 1981 and 1987). Confidence interval of the mixture and onset of death after mixture administration are not given (recommended in OECD TG 1981 and in OECD TG, 1987).
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered acceptable.

(Dates of exp. work: April 1983 to May 1983)

Materials and methods:

The aim of the study was to clarify whether additive or super-additive acute toxic affects occur after single or combined oral administration of cyfluthrin (FCR 1272) and methamidophos (SRA 5172). For this purpose, cyfluthrin (batch no.: 816170019, purity: 94.9 %) and methamidophos (batch no.: 808319101, purity: 73.0 %) were either given alone or simultaneously to unfed male rats (Wistar rats WISW [SPF-CBP], source: [redacted], 160-200 g).
A mixture of Cremophor EL and water served as vehicle. The application volume amounted to 1 mL/100 g bw.
Dosing schedule: 1-14-16-20-22.4*-23.6 mg/kg bw cyfluthrin (5 or 10* rats/dosage group), 1-10-14-16-20-25 mg/kg bw methamidophos (5 rats/dosage group), 14-20*-25*-28*-31.5*-33.5 mg/kg bw 52.94 % cyfluthrin and 47.05 % methamidophos (5 or 10* rats per dosage group).
The composition of cyfluthrin and methamidophos in the mixture was based on their equitoxic doses proportional to their LD_{50} values.
Recording period: 0-14 days.
Bw: Only at the beginning of the study.
Clinical signs were examined, necropsy was also performed.
Statistics: Calculation of median LD_{50} values according to the method of Rosiello, Essigmann and Wogan (1977). The theoretical LD_{50} value for the combination study was determined as published by Finney (1971) by means of the LD_{50} values for each substance and based on the assumption of an additive effect.

Results and discussions:

Table B. 6.8-13: Oral LD_{50} after 14 days in fasted, male rats after administration of cyfluthrin and/or methamidophos

Substance	NOAEL [mg/kg bw][#]	LD_{50} [mg/kg bw][*]
cyfluthrin	1	18 (16.9-20.7)
methamidophos	1	16 (14.2-19.5)
cyfluthrin + methamidophos	<14	26 (observed) 17 (expected)

= maximum dosage without clinical signs.
* = () confidence interval (95 %).

Onset of death: Within hours for cyfluthrin and methamidophos.
Clinical signs after administration of cyfluthrin were observed from a dose of 14 mg/kg bw onward. To the symptoms belonged disturbed behaviour, dyspnoe, salivation, digging and grooming movements, spread gait, rolling, temporary shaking, uncoordinated movements and vocalisation.
Clinial signs occurring after administration of methamidophos were apathy, bristling coat, convulsions, dacryohaemorrhoea and salivation. Only the lowest dose was associated with no clinical signs.
Disturbed behaviour, dyspnoea, reduced motility, convulsions, abnormal posture, salivation, dacryohaemorrhoea, spread and uncoordinated gait as well as soft faeces were observed after administration of the mixture. There was no dose associated without clinical signs.
The factor 0.65 between the calculated and the observed LD_{50} values indicate rather a sub-additive than a super-additive effect of the two substances.

Conclusion:

The study is acceptable. It provides additional information on the active ingredient.
No super-additive, but a sub-additive effect was observed after acute oral administration of equitoxic doses of cyfluthrin together with methamidophos was present.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results section were revised.
This acute oral toxicity study is generally in agreements with the criteria of OECD-Guideline no. 401 (adopted May 12, 1981). It was performed with fasted male rats.
There are some minor deviations from the guideline: Only one sex instead of both sexes per substance was used. Individual body weights and body weight development or individual signs with durations were not reported. Furthermore, the confidence interval of the mixture and onset of death after mixture administration are not given.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and methamidophos in cremophor EL/water were 18 mg/kg bw and 16 mg/kg bw, respectively. The observed LD_{50} value in the mixture was 26 mg/kg bw. From a concentration of 14 mg/kg bw cyfluthrin clinical signs occurred. To the signs observed belonged for example disturbed behaviour, dyspnoea and salivation. Oral administration of methamidophos also led to clinical signs like for example apathy, bristling coat and convulsions. Only the lowest dose did not result in clinical signs. Toxic symptoms after administration of the mixture occurred at all doses. The symptoms consisted for example of disturbed behaviour, dyspnoea and reduced motility.

Data point:	KCA 5.8 /17
Report:	[redacted] 1984, TOX9401948 FCR 1272 (Cyfluthrin), BOQ 5812315 (Propoxur) - study for combination toxicity - Report no.: 12544 (March 14, 1984); [redacted]
Guideline(s):	The test is similar to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Only one sex instead of both sexes per substance (demanded in OECD, TG 1981). No necropsy performed (deviation from recommendation in OECD TG 1981 and 1987). Neither clinical signs nor onset of death were recorded (recommended in OECD TG, 1981 and in OECD TG, 1987). Individual body weights and body weight development and duration of individual signs (to assess delayed effects) were not reported (recommended in OECD TG 1981 and 1987).

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered acceptable.

(Dates of exp. work: August 1983 to December 1983).

Materials and methods:

The aim of the study was to clarify whether additive acute toxic affects occur after single or combined oral administration of cyfluthrin (FCR 1272) and propoxur (BOQ 5812315). For this purpose, cyfluthrin (batch no.: 816270011, purity: 93.7 %)) and propoxur (batch no.: 234101303, purity: 99.3 %) were either given alone or simultaneously to unfed male rats (Wistar rats WISW [SPF-CBP], source: [redacted], 160-200 g).
A mixture of Cremophor EL and water served as vehicle. The application volume amounted to 1 mL/100 g bw.
Dosing schedule: 10-15-20-25 mg/kg bw cyfluthrin (5 rats/dosage group), 25*-35.5-50-60-71 mg/kg bw methamidophos (5 or 10* rats/dosage group), 53-56-63 mg/kg bw 31 % cyfluthrin and 69 % propoxur (5 rats per dosage group).
The composition of cyfluthrin and methamidophos in the mixture was based on their equitoxic doses proportional to their LD_{50} values.
Recording period: 0-14 days.
Bw: Only at the beginning of the study.
Clinical signs were not examined.
Statistics: Calculation of median LD_{50} values according to the method of Litchfiled and Wilcoxon (1949). The theoretical LD_{50} value was determined as published by Lorenz and Mueller (1967) and based on the individual LD_{50} values as well as the percentages present in the mixture.

Results and discussions:

Table B. 6.8-14: Oral LD_{50} after 14 days in fasted, male rats after administration of cyfluthrin and/or propoxur

Substance	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
cyfluthrin	not applicable.	20 (16-26)
propoxur	not applicable.	45 (35-58)
cyfluthrin + propoxur	not applicable.	57 (53-61, observed) 32.5 (expected)

= maximum dosage without clinical signs.
* = () most likely confidence interval (95 %), not specified in the report.

Onset of death: Not determined.
Clinical signs: Not determined.
The factor 0.6 between the calculated and the observed LD_{50} values indicate rather a sub-additive than a super-additive effect of the two substances.

Conclusion:

The study is acceptable. It provides additional information on the active ingredient.
No super-additive, but a sub-additive effect was observed after acute oral administration of equitoxic doses of cyfluthrin together with propoxur was present.

Re-evaluation by the RMS (2015):

The study is still considered acceptable. It should be noted that the materials and methods section as well as the results section were revised.

This acute oral toxicity study is similar to OECD-Guideline no. 401 (adopted May 12, 1981). It was performed with male rats.
There are some minor deviations from the guideline: Only one sex instead of both sexes per substance was used. Furthermore, neither necropsy findings, clinical signs nor onset of death were recorded.
Individual body weights and body weight development and duration of individual signs (to assess delayed effects) were not reported.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and propoxur in cremophor EL/water were 20 mg/kg bw and 45 mg/kg bw, respectively. The observed LD_{50} value in the mixture was 57 mg/kg bw.

Data point:	KCA 5.8 /18
Report:	[redacted] 1984, TOX9401949 FCR 1272 (Cyfluthrin), DDVP (Dichlorvos) - study for combination toxicity - Report no.: 12567 (March 27, 1984); [redacted]
Guideline(s):	The test is similar to OECD-Guideline no. 401 (1981) which complies to Directive 92/69/EEC method B 1.
Deviations:	Only one sex instead of both sexes per substance (demanded in OECD TG, 1981). No necropsy performed (deviation from recommendation in OECD TG 1981 and 1987). Neither clinical signs nor onset of death were recorded (recommended in OECD TG 1981 and in OECD TG 1987). Individual body weights and body weight development and duration of individual signs (to assess delayed effects) were not reported (recommended in OECD TG 1981 and 1987).
GLP:	When the study was performed, GLP was not compulsory.
Acceptability:	The study is considered acceptable.

(Dates of exp. work: August 1983 to December 1983).

Materials and methods:

The aim of the study was to clarify whether super-additive acute toxic affects occur after single or combined oral administration of cyfluthrin (FCR 1272) and dichlorvos (DDVP). For this purpose, cyfluthrin (batch no.: 816270011, purity: 93.7 %)) and DDVP (batch no.: 809236239, purity: 98.9 %) were either given alone or simultaneously to unfed male rats (Wistar rats WISW [SPF-CBP], source: [redacted], 160-200 g).
A mixture of Cremophor EL and water served as vehicle. The application volume amounted to 1 mL/100 g bw.
Dosing schedule: 10-15-20-25 mg/kg bw cyfluthrin (5 rats/dosage group), 35.5-37.5*-40-42.5*-45 mg/kg bw DDVP (5 or 10* rats/dosage group), 63-71-80 mg/kg bw 33 % cyfluthrin and 67 % DDVP (5 rats per dosage group).
The composition of cyfluthrin and DDVP in the mixture was based on their equitoxic doses proportional to their LD_{50} values.
Recording period: 0-14 days.
Bw: Only at the beginning of the study.
Clinical signs were not examined.
Statistics: Calculation of median LD_{50} values according to the method of Litchfiled and Wilcoxon (1949). The theoretical LD_{50} value was determined as published by Lorenz and Mueller (1967) and based on the individual LD_{50} values as well as the percentages present in the mixture.

Results and discussions:

Table B. 6.8-15: Oral LD_{50} after 14 days in fasted, male rats after administration of cyfluthrin and/or DDVP

Substance	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
cyfluthrin	not applicable.	20 (16-26)
DDVP	not applicable.	41 (39-42)
cyfluthrin + DDVP	not applicable.	70 (62-79, observed) 30.5 (expected)

= maximum dosage without clinical signs.
* = () most likely confidence interval (95 %), not specified in the report.

Onset of death: Not determined.
Clinical signs: Not determined.
The factor 0.4 between the calculated and the observed LD_{50} values indicate rather a sub-additive than a super-additive effect of the two substances.

Conclusion:

The study is acceptable. It provides additional information on the active ingredient.
No super-additive, but a sub-additive effect was observed after acute oral administration of equitoxic doses of cyfluthrin together with DDVP was present.

Re-evaluation by the RMS (2015):

The study is still considered to acceptable. It should be noted that the materials and methods section as well as the results section were revised.
This acute oral toxicity study meets basically the criteria of OECD-Guideline no. 401 (adopted May 12, 1981). It was performed with male rats.
There are some minor deviations from the guideline: Only one sex instead of both sexes per substance was used. Furthermore, neither necropsy, clinical signs nor onset of death were recorded.
Individual body weights and body weight development and duration of individual signs (to assess delayed effects) were not reported.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and DDVP in cremophor EL/water were 20 mg/kg bw and 41 mg/kg bw, respectively. The observed LD_{50} value in the mixture was 70 mg/kg bw.

Data point: KCA 5.8 /20

Report: [redacted] 1988, TOX9401950
E 6876 and FCR 1272 - study for combination toxicity to rats - Report no.: 16968 (July 28, 1988); [redacted]

Guideline(s): The test is based on OECD-Guideline no. 401 (1981).

Deviations: Only one sex instead of both sexes per substance (demanded in OECD TG, 1981).
Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1981 and 1987).
Individual body weights, individual pathological findings and duration of individual signs were not reported (recommended in OECD TG 1981 and 1987).
A method for the calculated LD_{50} value (combination study) is not given.

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered acceptable.

(Dates of exp. work: January 1987 to April 1987).

Materials and methods:

The aim of the study was to clarify whether potentiations of acute toxic affects occur after single or combined oral administration of cyfluthrin (FCR 1272) and omethoate (E 6876). For this purpose, cyfluthrin (batch no.: 233690489, purity: 95.7 %) and omethoate (batch no.: 233690604, purity: 95.5 %) were either given alone or simultaneously to unfed male rats (Wistar rats WISW [SPF-CBP], source: [redacted], 160-180 g).
PEG 400 served as vehicle. The application volume amounted to 5 mL/1000 g bw.
Dosing schedule: 400-450-500-630 mg/kg bw cyfluthrin (5 rats/dosage group), 25-31.5-35.5-40-50 mg/kg bw omethoate (5 rats/dosage group), 100-160-180-250-355-400 mg/kg bw 92.42 % cyfluthrin and 7.58 % omethoate (5 rats per dosage group). The composition of cyfluthrin and omethoate in the mixture was based on their percentage ratios of their LD_{50} values (equitoxic doses).
The fasted animals were fed 2 h after single dosing.
Recording period: 0-14 days.
Bw: Before administration and then weekly.
Clinical signs were examined, necropsy was also performed.
Statistics: Calculation of median LD_{50} values according to the method of Rosiello (1977) (modified by Pauluhn, 1983).

Results and discussions:

Table B. 6.8-16: Oral LD_{50} after 14 days in fasted, male rats after administration of cyfluthrin and/or omethoate

Substance	NOAEL [mg/kg bw][#]	LD_{50} [mg/kg bw][*]
cyfluthrin	<400	500 (441.2-567)
omethoate	<25	41 (35.7-47.4)
cyfluthrin + omethoate	<100	218(139.3-342.2, observed) 270 (expected)

= maximum dosage without clinical signs.
* = () confidence interval (95 %).

Onset of death: Within hours until 5 days for cyfluthrin, between 41 min and 2 h for omethoate and between 1 h and 1 day for the mixture.
Clinical signs after administration of cyfluthrin were observed from a dose of 400 mg/kg bw onward. To the symptoms belonged increased salivation, increased motility, uncoordinated or spread gait and apathy. At higher doses also soft stool or diarrhoea, bristling coats, digging and grooming movements and vocalisation (630 mg/kg bw) were observed. Clinical signs were observed at the earliest after 1 h disappeared after a maximum of seven days.
Clinial signs after administration of omethoate occurred from 25 mg/kg bw. To the symptoms belonged palmospasms, dyspnoea, dacryohaemorrhoea, apathy and soft stool. At higher doses shortness of breath, spastic gait, increased salivation, reduced motility and bristling coats occurred. The symptoms were observed not before 25 min and lasted up to a maximum of 7 days.
After administration of the mixture clinical signs were observed from a dose of 100 mg/kg bw. To these signs belonged palmospasms, dyspnoea, increased salivation, uncoordinated or spread gait and apathy. At higher doses reduced motility, diarrhoea and apathy were noted. Spastic gait, soft stool, dacryohaemorrhoea, bristling coats and prostration on stomach occurred only after a dose of 180 mg/kg bw. The clinical signs started at the earliest after 26 min and lasted up to a maximum of 5 days. The factor 1.24 between the calculated and the observed LD_{50} values indicate a slight super-additive (potentiating) combination effect.

Body weight:
Omethoate: A treatment-induced temporary effect for 50 mg/kg bw group (not by the end of observation period)
Cyfluthrin: No effect.
Mixture: No effect
Gross pathology:
Omethoate: Animals dying during observation: lung: patchy, distended; spleen: patchy, pale; glandular stomach: sporadically reddened; Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ alterations.
Cyfluthrin: Animals dying during observation: lung: patchy, sometimes slightly distended; liver: sporadically patchy; spleen and kidneys: sporadically patchy, slightly pale, sporadically kidney structure faint; sporadically stomach distended; glandular stomach: sporadically slightly reddened; small intestine: sporadically containing yellow mucous contents. Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ alterations.
Mixture: Animals dying during observation: lung: patchy, slightly distended, sporadically dark; liver: sporadically patchy; spleen: patchy, pale; kidney: structure sporadically faint; stomach: sporadically distended; glandular stomach: sporadically reddened; small intestine: sporadically distended. Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ alterations.

Conclusion:

The study is acceptable. It provides additional information on the active ingredient.
No strong super-additive effect was observed after acute oral administration of equitoxic doses of cyfluthrin together with omethoate was present.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results section were revised.
This acute oral toxicity study is based on OECD-Guideline no. 401 (adopted May 12, 1981). It was performed with male rats.
There are some minor deviations (no impact on acceptability status):
Only one sex instead of both sexes per substance was used. Food was withheld for 2 h instead of 3-4 h after substance administration. Individual body weights, individual pathological findings and duration of individual signs were not reported. Finally, a method for the calculated LD_{50} value (combination study) is not given.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and omethoate in PEG 400 were 500 mg/kg bw and 41 mg/kg bw, respectively. The observed LD_{50} value in the mixture was 218 mg/kg bw.

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin by the RMS in May, 2002 (ASB2014-9599):

Data point:	KCA 5.8 /19
Report:	[redacted] 1994, TOX2001-1764 NTN 33893 (c.n. Imidacloprid [proposed]), FCR 1272 (c.n. Cyfluthrin) – Study for combination toxicity in rats, Report No. 23420, Study Nos. T 8055008, T 7055007, T 0055109, unpublished; [redacted] NTN 33893 (c.n. Imidacloprid (proposed)), FCR 1272 (c.n. Cyfluthrin) – Study for combination toxicity in rats – Addendum to Report No. 23420; Report-No. 23420 A, unpublished; [redacted]

Guideline(s):	The study was based on OECD Guideline No. 401 (adopted 24 February 1987)
Deviations:	Only 2 instead of 3 doses tested for cyfluthrin and the mixture (recommended in OECD TG, 1987) Food was withheld for 2 h instead of 3-4 h after substance administration (recommended in OECD TG, 1987).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is considered supplementary.

(Dates of exp. work: June 1993 to August 1993).

Materials and methods:

Test material: Imidacloprid (NTN 33893), purity: 97.6 %, batch no.: 816255037 and cyfluthrin (FCR 1272), purity: 95.1 %, batch no.: 238005176.
Test animals: male SPF-bred Wistar rats (HSD/WIN:WU).
Source: [redacted].
Body weight at the start of the study: 164-190 g
The pair of active ingredients imidacloprid and cyfluthrin was examined for (subadditive, additive or superadditive) combination effects in male rats by the oral route of administration.
The test substances were formulated in deionised water using 2 % (v/v) Cremphor®EL. The treatment volume was 10 mL/kg bw.
Dosing schedule: 14-20 mg/kg bw cyfluthrin (5 rats/dosage group), 500-600-750 mg/kg bw imidacloprid (5 rats/dosage group), 315-450 mg/kg bw 97.33 % imidacloprid and 2.67 % cyfluthrin (5 rats/dosage group).
The fasted animals were fed 2 h after single dosing.
Recording period: 0-14 days.
Bw: One day before treatment and on days 4, 8 and 15.
Clinical signs were examined, necropsy was also performed.
Statistics: Calculation of median LD_{50} values according to the method of Bliss (1935), in the form published by Rosiello et al. (1977) and Baird et al. (1979). An approximate LD_{50} value is given if the rate of mortality was greater than 0 and less than 100 % in only two of the dose groups and at least one dose caused 0 or 100 % mortality.
The lethality curves of the two separate test substances, and of their combination at the indicated mixing ratio, were prepared on the basis of the test results. In addition, the LD_{50} expected for the combination at the indicated mixing ratio was calculated from the results for the separate test substances. The following algorithm was used to calculate the theoretical LD_{50}:

$$\sum_{i=n}^{1} \frac{\text{Purity}_i \text{ (in percent)}}{LD_{50_i} * 100} = \frac{1}{\text{Calculated } LD_{50}}$$

The (additive, subadditive or superadditive) combination effect was estimated by comparing the experimentally determined with the calculated values for the LD_{50}. This was accomplished by dividing the calculated LD_{50} by the experimentally determined LD_{50} for the combination to calculate a factor, which was then used to assess whether a straight additive (factor approx. 1) or a superadditive combination effect (factor >1) was present. Since variations occur in determinations of the LD_{50}, a range from 0.8 to 1.2 is specified for an additive effect. True subadditive or superadditive effects are only present when the factor is smaller or larger.

Results and discussions:

Imidacloprid was found to exhibit moderate/low toxicity, cyfluthrin to be highly toxic, and the combination of imidacloprid and cyfluthrin to be moderately toxic in male rats by the acute oral route of administration. The corresponding LD_{50} values and clinical findings are summarised in the following table.

Table B. 6.8-17: Combination acute oral toxicity: Summary of results

Test substance Median lethal dose* NOAEL#	Clinical signs and death
Cyfluthrin LD_{50}: approx. 15 mg/kg bw NOAEL: <14 mg/kg bw	The main toxic signs were apathy, laboured breathing, decreased motility, uncoordinated movements, broad gait, digging activities, salivation and lacrimation, red secretion at the orbital margins, transient rolling over and shaking, and transient vocalisation. Mortalities occurred at doses of 14 mg/kg bw and above. Duration of signs: from 34 min to 2 days. Time of death: between 1,5 h and 2 h
Imidacloprid LD_{50}: approx. 547 mg/kg bw NOAEL: <500 mg/kg bw	The main toxic signs were apathy, decreased motility, laboured and/or accelerated breathing, staggering gait, spasmodic state, narrowed palpebral fissures, hairless parts of the body well supplied with blood and transient tremor of the head as well as transient convulsions and tonical cramps. Mortalities occurred at doses of 500 mg/kg bw and above. Duration of signs: from 27 min to 3 days. Time of death: between 2,5 h and 2 days
Cyfluthin and imidacloprid LD_{50}: approx. 414 mg/kg bw NOAEL: <315 mg/kg bw (calculated LD_{50}: 281 mg/kg bw)	The main toxic signs observed when the test substances were combined were apathy, piloerection, laboured and/or accelerated breathing, decreased motility, staggering gait, spasmodic state, uncoordinated movements, salivation, digging activities, narrowed palpebral fissures, hairless parts of the body well supplied with blood, transient shaking and tremor of the head, and transient grooming activities. Mortalities occurred at doses of 315 mg/kg bw and above. Duration of signs: from 30 min to 2 days. Time of death: 1 day

* = no confidence interval (95 %) in this case possible.
# = maximum dosage without clinical signs.

Clinical signs were observed in all dosage groups.
Body weight: Retarded body weight development was only observed in one animal (600 mg/kg bw imidacloprid).
Gross pathology (salient features):
Animals dying during observation - imidacloprid: thymus dark red in places; lungs incompletely collapsed, dark red; liver dark red in places, pale in places, distinct lobulation; spleen pale, dark red in places; both kidneys pale, dark red in places; renal cortex red; glandular stomach exhibits light-coloured, creamy deposits.
Animals dying during observation – cyfluthrin: lungs incompletely collapsed; liver dark red; spleen moderately pale; both kidneys moderately pale in isolated cases.
Animals dying during observation – mixture: lungs dark red, incompletely collapsed; liver dark red in places; spleen pale; both kidneys pale; renal cortex dark red; pancreas white.
Animals sacrificed at the end of the observation period: No evidence for substance-related, macroscopically apparent changes.
Comparison of the separate active ingredients with their combination showed the combination to induce no clinical or gross pathological findings of greater severity. A subadditive effect for the present combination of active ingredients was established on the basis of these study results.
A theoretical $LD_{50:}$ of 281 mg/kg bw was calculated for the test substance consisting of an equitoxic combination of imidacloprid and cyfluthrin. The experimentally determined value is 414 mg/kg bw. Using the described evaluation method for the combination effect, the present combination of test substances was found to exert a subadditive effect, since the calculated factor was 0.7 in this case.

Conclusion:

Under the conditions of the study, the experimental results afforded evidence for a subadditive combination effect by the examined test substances cyfluthrin and imidacloprid.

Re-evaluation by the RMS (2015):

The study is now considered to be supplementary. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be acceptable. However, due to some critical points listed below the study is now longer considered acceptable. It should be noted that the materials and methods section as well as the results section were revised.
This acute oral toxicity study is generally in agreements with the criteria of OECD-Guideline no. 401 (adopted February 24, 1987). It was performed with male rats.
There are some deviations from the guideline:
Only 2 instead of 3 doses tested for cyfluthrin and the mixture. Food was withheld for 2 h instead of 3-4 h after substance administration.
Under the conditions of the study and based on the information given in the report, the LD_{50} values for cyfluthrin and imidacloprid in cremophor EL/water were approximately 15 mg/kg bw and 547 mg/kg bw, respectively. The observed LD_{50} value in the mixture was approximately 414 mg/kg bw. From a concentration of 14 mg/kg bw cyfluthrin clinical signs occurred. To the signs observed belonged for example apathy, laboured breathing and decreased motility. Oral administration of imidacloprid also led to clinical signs like for example apathy, decreased motility, laboured and/or accelerated breathing. There was no dose associated with no clinical signs. Toxic symptoms after administration of the mixture occurred at all doses. The symptoms consisted for example of apathy, piloerection, laboured and/or accelerated breathing.

B.6.8.2.4 Study on tumour promotion

Publications evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.8 /21
Publication	[redacted] Cell Biology and Toxicology 5 (1989), 67-75: Effects of tetradecanoyl phorbol acetate, pyrethroids and DDT in the V79, TOX9401951
Guideline(s):	Not applicable.
Deviations:	The validity of this test is not fully established.
GLP:	Not applicable.
Acceptability:	This publication is considered supplementary.

(dates of exp. work: not given).

Conclusion:

This test was performed with V79 fibroblasts. The aim was to examine the inhibition of communication between cells in culture in order to derive information on tumour-promoting activity. Cyfluthrin (analytical grade, purchased from Firma S. Ehrenstorfer [Augsburg, FRG]) was found not to inhibit intercellular communication up to 15 µM. Therefore, it has been suggested that cyfluthrin has no tumour-promoting potential.

Re-evaluation by the RMS (2015):

The study is still considered to be supplementary. It should be noted that the conclusion was revised. Under the conditions of the study cyfluthrin did not inhibit intercellular communication in non-cytotoxic concentrations up to 15 µM. Hence, it seems to have no tumour-promoting potential *in vitro*. However, the information value of this study is questionable as the validity of the test is not fully established.

B.6.8.2.5 Mechanistic studies (cyfluthrin)

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin by the RMS in May, 2002 (ASB2014-9599):

Data point:	KCA 5.2.3 /03
Report:	[redacted] 1996, TOX2001-1767 Determination of Cyfluthrin (FCR 1272) in Serum, Fat and Brain of Rats after Inhalation Exposure or Oral Administration - Analytical Part of Study T7058167 Study-No. P65345012, Report-No. MR-365/95, unpublished [redacted]
Guideline(s):	No guideline exists for this type of investigation.
Deviations:	Details on the study design or analytical methods were not given in the study report. There is a lack of data concerning the animals (strain and source).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is considered supplementary.

(Dates of experimental work: September 1994 to February 1995).

Materials and methods:

Test material: Cyfluthrin (batch no. 910420ELB09, purity: 94.5 %)
Test animals: Male rats, strain and source unclear.
A study on the kinetic profile of cyfluthrin in male rats following single inhalation exposure of 38.7 mg cyfluthrin/m³ air for six hours or single oral treatment with 9 mg cyfluthrin/kg bw was conducted. Each one group served as control. Samples of serum, omental fat and brain were taken from 20 rats/group after 0–0.5–2.5–3.5–18–20 hours after end of exposure or after application, respectively.
Samples of serum, omental fat and brain were taken on each sampling time point. Serum was mixed with water and given on an Extrelut cartridge. Elution of cyfluthrin was performed with ethyl acetate. Following cleanup by chromatography on silica gel, a mixture of n-hexane and dichloromethane served for elution. Cyfluthrin was determined by gas chromatography with an electron capture detector (ECD). Fat and brain were extracted with acetonitrile and partitioned against n-hexane. After further cleanup with gel permeation chromatography (GPC), silica gel column and Cig-cartridge, cyfluthrin was determined by gas chromatography (with ECD).

Results and discussions:

Table B. 6.8-18: Mean cyfluthrin concentration in serum, fat and brain after inhalation and oral gavage exposure

	concentration of cyfluthrin							
	inhalation				oral gavage			
Sampling time [h]	control	0–0.5	2.5–3.5	18–20	control	0–0.5	2.5–3.5	18–20
serum (mg/L)	<0.01[a]	0.062	0.011	<0.01[a]	<0.01	0.022	0.146	<0.01[a]
fat (mg/kg)	<0.005[b]	0.652	0.819	0.718	<0.005	0.599	1.39	1.18
brain (mg/kg)	<0.005[b]	0.071	0.026	<0.005[b]	<0.005	0.010	0.038	0.007

[a] 0.01 mg/L = limit of quantification for serum.
[b] 0.005 mg/L = limit of quantification for fat and brain.

Conclusion:

No conclusion given.

Re-evaluation by the RMS (2015):

The study is still considered to be supplementary. It should be noted that the materials and methods section was revised.
Further details on the study design, analytical methods or animals (strain and source) were not given in the study report. A reference to Study-No. T7058167 is mentioned in the study report. However, this document is not available to the RMS.

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin by the RMS in May, 2002 (ASB2014-9599):

Data point: KCA 5.2.1 /14

Report: [redacted] 1996, TOX2001-1768
Cyfluthrin: Concentration of the Parent Compound in Blood Plasma, Brain and Omental Fat of Rats Following Administration with the Feed or by Oral Administration
Study-No. M 182 0669-2, Report-No. MR-625/95, unpublished
[redacted]
(Experimental work from 29 August 1994– 22 February 1995)

Guideline(s): No specific guideline is available for this kind of study.

Deviations: Details on the analytical methods were not available.

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is considered acceptable.

(Dates of experimental work: August 1994 to February 1995).

Materials and methods:

Test material: Cyfluthrin, purity: 94.5 %, batch no.: 910420ELB09 (working standard).
Test animals: Male Wistar rats (Hsd/Win: WU); age: 9–10 weeks Source: [redacted]

In context with the neurotoxicity of pyrethroids in animals under discussion, especially in new-born rats, it was found necessary primarily to determine the concentration of cyfluthrin in blood plasma, brain and fat of adult rats in dependence on the route of exposure in order to establish a database for the conduct of further studies. In test no. 1, two groups of 10 rats each received the compound in pulverised Altromin standard feed at a target concentration of 90 ppm for a period of 72 hours. In test no. 2, two groups of 10 rats each received the compound by gavage in polyethylene glycol solution in four oral doses at target levels of 9 mg/kg bw, one dose/day on four consecutive days in time intervals of 24 hours. In both tests, one group was sacrificed at 3 hours, the other group at 19 hours after the end of treatment. Brain, omental fat and blood plasma was prepared. The concentration of the parent compound was determined after pooling the respective tissues of each animal group and extraction procedures.
Note concerning the feeding experiment (90 ppm):
Group 1: A mean compound intake of 8.5 mg/kg bw/ day was calculated from mean feed consumption (26 g/day per animal) and mean body weight (276 g).
Group 2: A mean compound intake of 7.1 mg/kg bw/ day was calculated from mean feed consumption (22.3 g/day per animal) and mean body weight (282 g).
Samples of serum, omental fat and brain were taken on each sampling time point. Serum was mixed with water and given on an Extrelut cartridge. Elution of cyfluthrin was performed with ethyl acetate. Following cleanup by chromatography on silica gel, a mixture of n-hexane and dichloromethane served for elution. Cyfluthrin was determined by gas chromatography with an electron capture detector (ECD). Fat and brain were extracted with acetonitrile and partitioned against n-hexane. After further cleanup with gel permeation chromatography (GPC), silica gel column and C18-cartridge, cyfluthrin was determined by gas chromatography (with ECD).

Results and discussions:

Table B. 6.8-19: Time course of cyfluthrin concentrations in serum, fat and brain after gavage or dietary administration to rats

	Concentration of cyfluthrin			
	Feed (90 ppm, 72 h exposure)		Gavage (9 mg/kg bw/d, 4 d exposure)	
Sampling time	3 h	19 h	3 h	19 h
Serum (mg/L)	0.019	<0.01[a]	0.257	<0.01[a]
Fat (mg/kg)	2.67	1.6	2.15	2.08
Brain (mg/kg)	0.014	<0.005[b]	0.06	<0.005[b]

[a] 0.01 mg/L = limit of quantification for serum.
[b] 0.005 mg/L = limit of quantification for fat and brain.

By both administration regimes, cyfluthrin was detectable in serum and in brain tissue only at the initial 3-h time point of investigation. Concentration levels were 13.5-fold higher in serum and 4.3-fold higher in brain after gavage treatment than after continuous exposure via the feed, based on approx. the same test substance intake (in mg/kg bw/d).
By far the highest cyfluthrin concentrations were found in fat and they were comparable in both tests. As to be expected, the residues in serum after 3 hours were much higher after gavage than by dietary exposure, because in gavage rats 9 mg/kg body weight was applied as a bolus dose 3 hours before sacrifice whereas the dietary intake of cyfluthrin happened continuously throughout the whole day. A comparison of the concentrations in brain obtained at 3 h and 19 h after the end of feeding period or last dosage distinctly indicated that the parent compound was rapidly eliminated from this tissue after both methods of exposure.

Conclusion:

No conclusion given.

Re-evaluation by the RMS (2015):

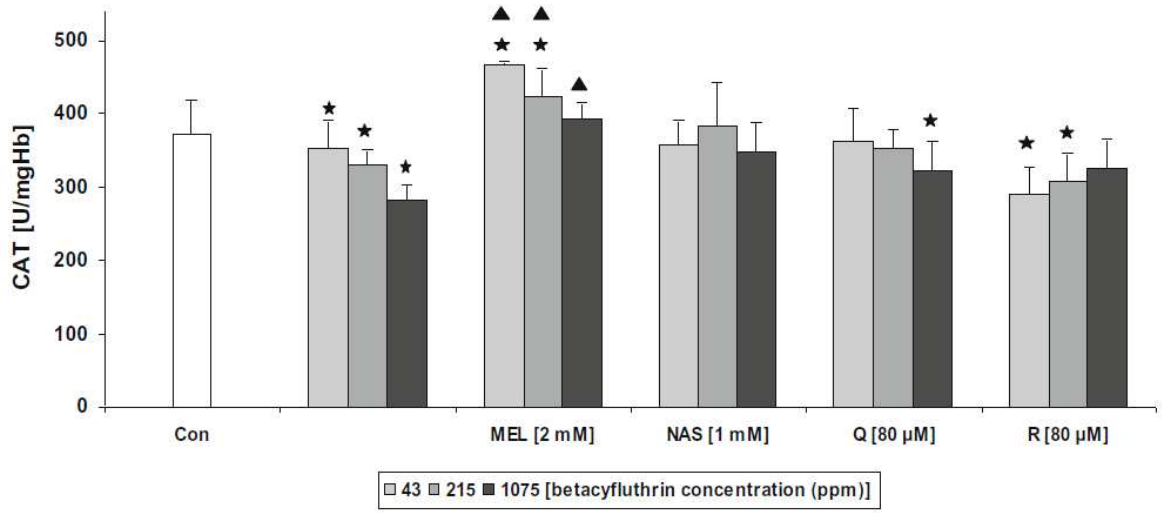
The study is still considered to be acceptable. It should be noted that the materials and methods section was revised.
Further details on the analytical methods are not given in the study report.

Further studies (literature search for cyfluthrin and beta-cyfluthrin)

Publication:	Sadowska-Woda, I. *et al.*, 2010, ASB2015-790 Effect of selected antioxidants in β-cyfluthrin-induced oxidative stress in human erythrocytes *in vitro*, Toxicology *in vitro* (24), 879-884
Deviations:	The data of the significance test are unclear
GLP:	Not applicable.
Acceptability:	The publication is considered acceptable.

(dates of exp. work: not given).

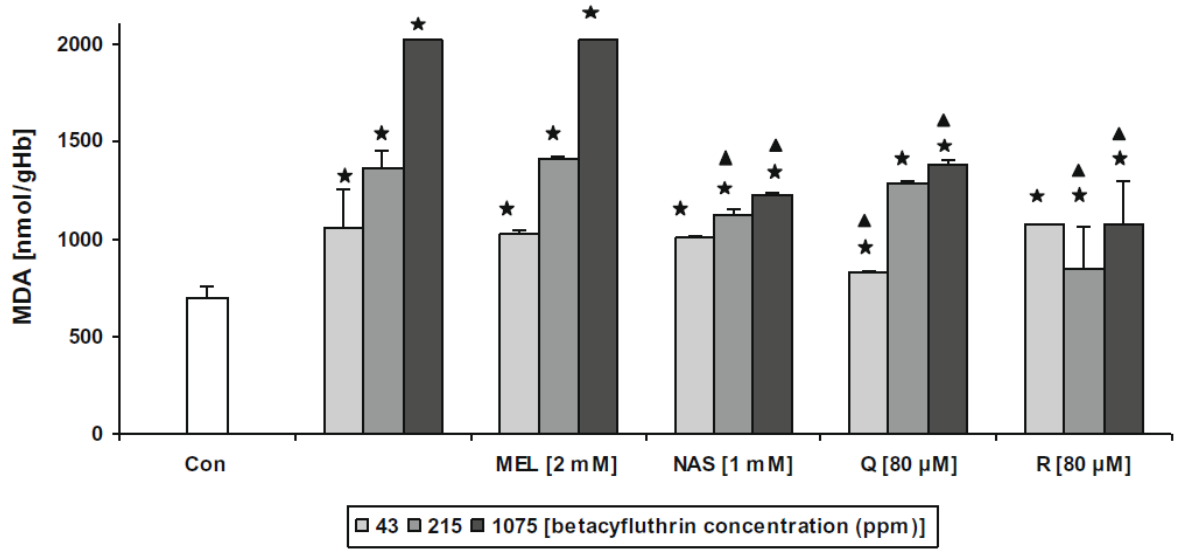
Summary:
The aim of the study was to investigate whether beta-cyfluthrin induces oxidative stress in human erythrocytes *in vitro*. Furthermore, the impact of beta-cyfluthrin on catalase (CAT) and superoxide dismutase (SOD) activity was studied. Finally, the experimenters researched the relevance of melatonin (MEL), *N*-acetylserotonin (NAS), quercetin (Q) and rutin (R) regarding beta-cyfluthrin-mediated cytotoxic effects.
Blood samples from three healthy donors (women, 21 or 31 years old) were taken to receive erythrocytes. Erythrocytes were either exposed to beta-cyfluthrin (0, 43, 215 and 1075 ppm; product number 46003-250MG, Sigma-Aldrich Co, Poland; solvent for stock solution: 20 % ethanol) for 4h at 37 °C or first preincubated with 2 mM MEL, 1 mM NAS, 80 µM Q or 80 µM R (all antioxidants dissolved in water) for 30 min followed by incubation with beta-cyfluthrin for 4 h. Afterwards, malondialdehyde concentrations, CAT and SOD activities and further haemolysis percentage (H) were measured.
CAT activities were significantly reduced at all beta-cyfluthrin concentrations (see Figure B.6.8-1). NAS seemed to maintain the activity better at control levels in comparison to the other antioxidants.



Data represents the mean +/- SD of six measurements from six independent experiments. Asterisk (*) indicates significant effect of different treatments compared to the control value; triangle (▲) indicates significant effect of antioxidant-pretreated erythrocytes compared to the erythrocytes exposed to beta-cyfluthrin only (Kruskal-Wallis test, p <=0.001).

Figure B.6.8-1: CAT activity of human erythrocytes incubated (4 h) with beta-cyfluthrin, MEL, NAS, Q or R plus different concentration of beta-cyfluthrin (pre-treatment times 30 min).

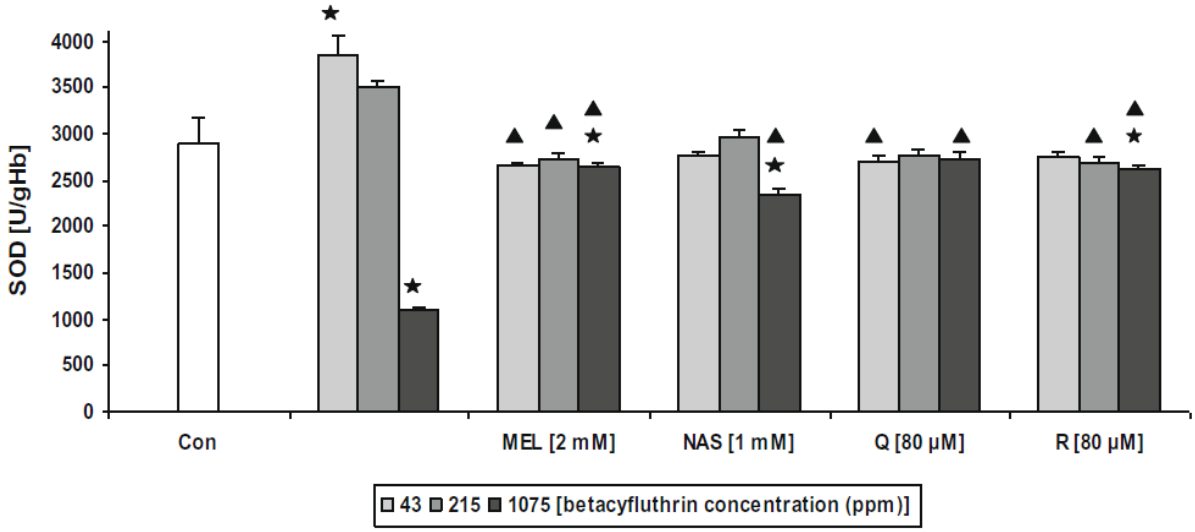
MDA levels were significantly and dose-related increased at all beta-cyfluthrin concentrations in comparison to the control (see Figure B.6.8-2). Pretreatment with the selected antioxidants resulted in significant elevated MDA in comparison to the control. MEL pretreatment did not affect the MDA induction by beta-cyfluthrin whereas the other substances led to reduced MDA levels (even though MDA induction was not prevented).



Data represents the mean +/- SD of six measurements from six independent experiments. Asterisk (*) indicates significant effect of different treatments compared to the control value; triangle (▲) indicates significant effect of antioxidant-pretreated erythrocytes compared to the erythrocytes exposed to beta-cyfluthrin only (Kruskal-Wallis test, p <=0.001).

Figure B.6.8-2: The alterations in MDA levels of human erythroxytes incubated (4 h) with beta-cyfluthrin, MEL, NAS, Q or R plus different concentration of beta-cyfluthrin (pretreatment times 30 min).

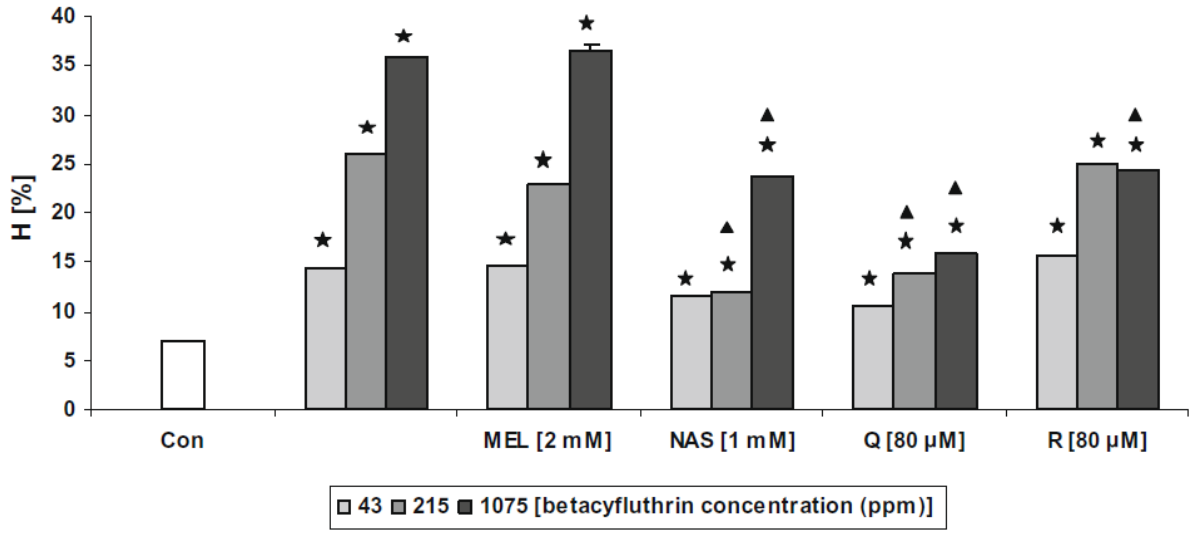
SOD activities were only significantly reduced at the highest beta-cyfluthrin dose applied (see Figure B.6.8-3), but significantly increased at the lowest dose in comparison to the controls.



Data represents the mean +/- SD of six measurements from six independent experiments. Asterisk (*) indicates significant effect of different treatments compared to the control value; triangle (▲) indicates significant effect of antioxidant-pretreated erythrocytes compared to the erythrocytes exposed to beta-cyfluthrin only (Kruskal-Wallis test, p <=0.001).

Figure B.6.8-3: SOD activity of human erythroxytes incubated (4 h) with beta-cyfluthrin, MEL, NAS, Q or R plus different concentration of beta-cyfluthrin (pretreatment times 30 min).

An amount of 15 or 35 % haemolysis was observed at the lowest or highest concentration of beta-cyfluthrin, respectively (Fig. 4). MEL pretreatment did not affect the induction of haemolysis by beta-cyfluthrin whereas the other antioxidants reduced the level of haemolysis (even though induction was not prevented).



Data represents the mean +/- SD of six measurements from six independent experiments. Asterisk (*) indicates significant effect of different treatments compared to the control value; triangle (□) indicates significant effect of antioxidant-pretreated erythrocytes compared to the erythrocytes exposed to beta-cyfluthrin only (Kruskal-Wallis test, p <=0.001).

Figure B.6.8-4: Haemolysis (%H) determinations of human erythroxytes incubated (4 h) with beta-cyfluthrin, MEL, NAS, Q or R plus different concentration of beta-cyfluthrin (pretreatment times 30 min).

In summary, treatment of erythrocytes with beta-cyfluthrin induced lipid peroxidation (indicated by higher MDA levels) and modified the antioxidant system. This supported the hypothesis that reactive oxygen species are involved in the toxic effects mediated by beta-cyfluthrin.

Conclusion:

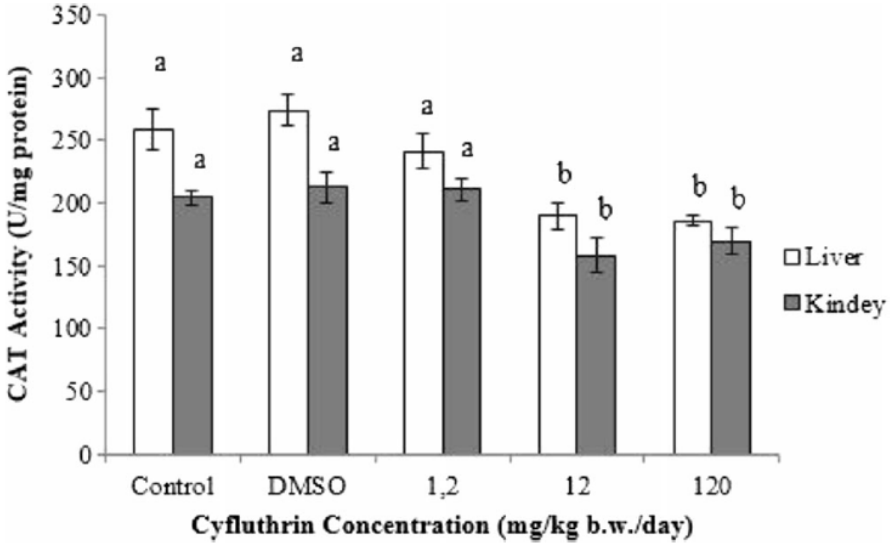
The study is considered acceptable. Under the conditions used in the study and based on the information given in the report, beta-cyfluthrin may be associated with oxidative stress *in vitro*. Furthermore, the antioxidant system is disturbed indicated by modified antioxidant enzyme activities.
It is noted that bars in the diagrams are marked with symbols reflecting significance even though there is an overlap of error bars. As no single data are presented, a recalculation is not possible.

The applicant stated that this study is not part of the data requirements as specified in Regulation (EU) No 283/2013. They are not necessary to further clarify the observed effects. However, this study provides additional information and is therefore reported.

Data point:	KCA 5.8
Publication:	Yilmaz, M. et al., 2014, ASB2015-888 The effects of cyfluthrin on some biomarkers in the liver and kidney of Wistar rats, Environ Sci Pollut Res (in press, DOI 10.1007/s11356-014-3734-6)
Deviations:	None.
GLP:	Not applicable.
Acceptability:	The publication is considered acceptable.

(dates of exp. work: not given).

Summary:
The aim of the study was to investigate the impact of cyfluthrin on the malondialdehyde (MDA) level as well as on the catalase (CAT) glutathione peroxidise (GPx) and acetylcholinesaterase (AChE) activities *in vivo*. For this purpose Wistar albino Sprague Dawley rats were treated with 1.2, 12 and 120 mg/kg bw/day cyfluthrin (CAS no.: 68359-37-5, Bayer Turkey, purity: 96 %, by intraperitoneally injection for 21 days. Then, rats were dissected, livers and kidneys were taken and biomarkers were analysed. Serum physiologic and the solvent used for cyfluthrin served as controls.
The CAT activity in the liver and kidney was only significantly decreased at the higher doses (see Figure B.6.8-5).



Letters a, b, and c are used to show significant differences between statistically compared groups. Data with the same letter indicate that there is no significant ($p>0.05$) difference between compared groups, while data with different letters indicate that there is a significant difference ($p<0.05$) between compared groups.

Figure B.6.8-5: Effects of cyfluthrin on CAT-specific activity in the liver and kidney of rats. Data are given as mean +/- standard error. The liver and kidney were treated individually in statistical analyses.

The GPx activity was significantly decreased at all doses in kidney and liver (see Figure B.6.8-6).

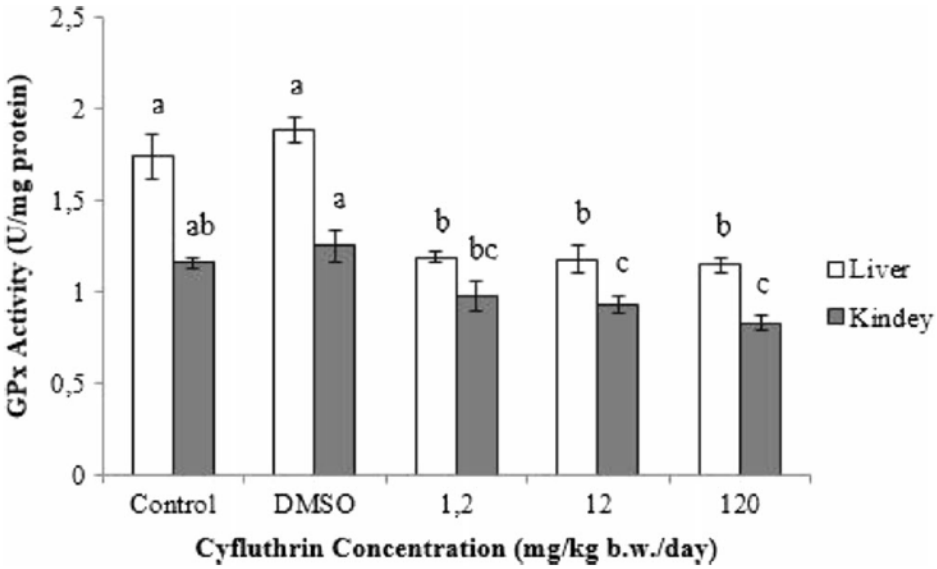


Figure B.6.8-6: Effects of cyfluthrin on GPx-specific activity in the liver and kidney of rats (U/mg protein). See Figure B.6.8-5 for details

The hepatic MDA levels of the rats were increased at all doses (see Figure B.6.8-7).

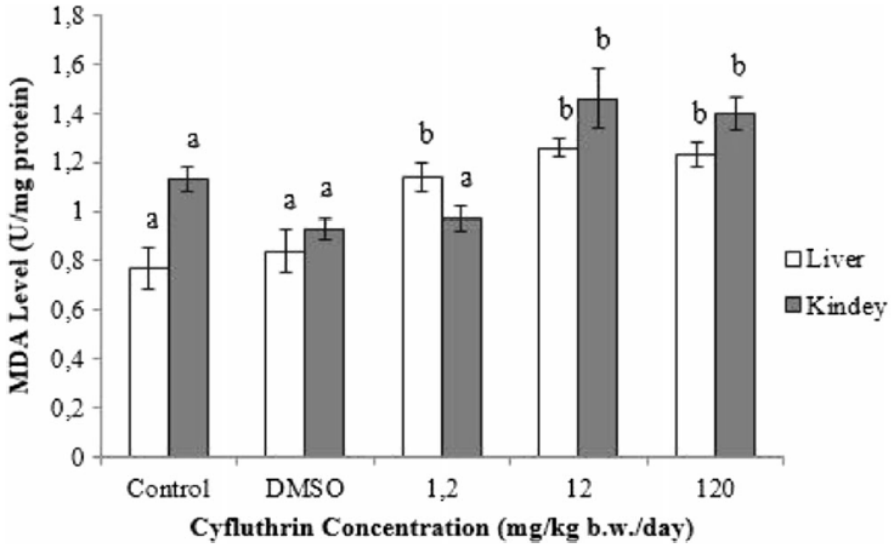


Figure B.6.8-7: Effects of cyfluthrin on on MDA level in the liver and kidney of rats (U/mg protein). See Figure B.6.8-5 for details

The AChE activity was significantly decreased in the livers at all doses (see Figure B.6.8-8). For the kidney a significant decrease was only observed at the highest dose applied.

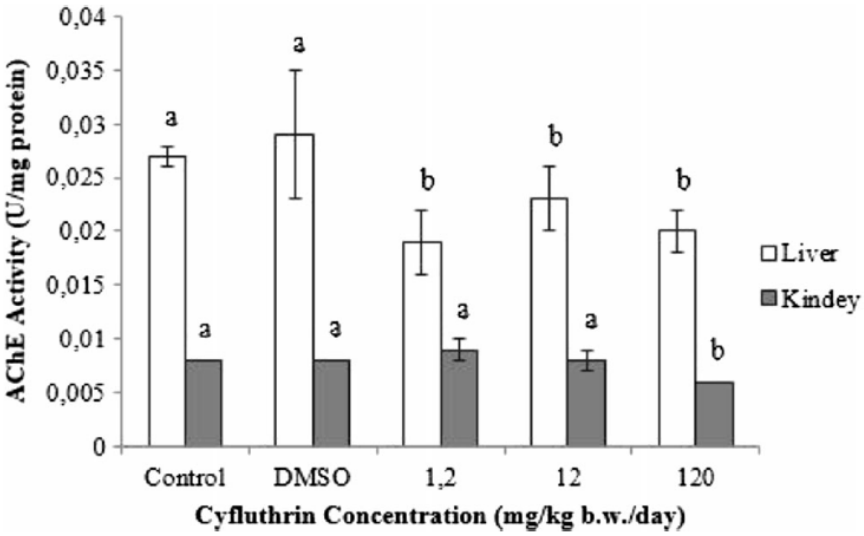


Figure B.6.8-8: Effects of cyfluthrin on AChE-specific activity in the liver and kidney of rats (U/mg protein). See Figure B.6.8-5 for details

Taken together, cyfluthrin resulted in a reduced renal and hepatic enzyme activity whereas the level of MDA was increased. Therefore it was concluded that cyfluthrin might damage the renal and hepatic antioxidant defence system in rats.

Conclusion:

The study is considered acceptable. Under the conditions used in the study and based on the information given in the report, cyfluthrin may be associated with a disturbance of the antioxidant defence system in the liver and kidney of rats.

The applicant stated that this study is not part of the data requirements as specified in Regulation (EU) No 283/2013. They are not necessary to further clarify the observed effects. However, this study provides additional information and is therefore reported.

B.6.8.2.6 Risk of Indoor use

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin by the RMS in May, 2002 (ASB2014-9599):

Data point:	KCA 5.8 /24
Publication:	Pauluhn, J. 1996, TOX2001-880 Risk assessment of pyrethroids following indoor use, Toxicology Letters 88, 339–348 (published)
Guideline(s):	Not applicable.
Deviations:	Some details regarding the study design (*e.g.* inhalable particle content, sex of mice) are not given.
GLP:	Not applicable.
Acceptability:	This publication is considered supplementary.

(dates of exp. work: not given).

Summary of the publication (addendum 1 2002, ASB2014-9599):
One notable form of toxicity associated with exposure to high concentrations of synthetic pyrethroids has been a cutaneous paraesthesia. This strong excitatory action on the sense organs in the vertebrate skin and upper respiratory tract is characteristic of synthetic pyrethroids, whereas the cyano-pyrethroids evoke more intense neuroexcitatory activities than the noncyano-pyrethroids. Such facial sensations and irritation symptoms appear to be produced by direct stimulation of peripheral sensory nerve endings rather than by inflammatory mechanisms.
Effects related to sensory irritation can be evoked by a wide variety of substances occurring in the indoor environment, and analysis of the etiopathological relationships presents difficult and complex medical and scientific issues. For the appropriate assessment of pyrethroids in the indoor environment, it would be helpful to have an objective laboratory assay to confirm and quantitate the degree of sensory irritation evoked by airborne pyrethroids.
A bioassay was established using the nociceptive system of mice and rats to assess the extent of pyrethroid-related sensory irritation to the respiratory tract. For analysis, aerosolised cyfluthrin (purity 93 %) was selected due to the greater potency of the α-cyano pyrethroids to evoke sensory irritation. Additionally, this pyrethroid was tested in a carpet-model to assess the extent to which pyrethroid-laden dust from carpets is likely to become airborne following continuous brushing. Comparative evaluations of the sensory irritation potential of aerosolised cyfluthrin in mice and rats revealed that for assessment of the sensory irritant threshold concentration, rats appeared to be more susceptible than mice. Measurements performed repeatedly during subacute exposure to the pyrethroid (6 h/day, 5 days/week for 4 consecutive weeks) did not indicate any alteration in responsiveness, and the magnitude of changes in breathing patterns was similar to those observed following acute l-h exposure. These findings confirm the conclusion that α-cyano-pyrethroids appear to act as "pure" sensory irritants and that the effects observed are non-cumulative and transient in nature. Concomitant respiratory tract inflammation and ensuing changes in susceptibility-common findings in chemical sensory irritants-did not occur. From the studies addressing the dislodgeability of pyrethroid containing dust from carpets, it is apparent that measurement of deposited dust is a poor substitute for airborne dust. Even under worst-case testing conditions (continuous brushing of the carpet for approximately l9 h in a bias-flow compartment), only a very small fraction of the pyrethroid laden dust particles charged to the carpet could be recovered airborne (0.04 %/m^2 per h).

Re-evaluation by the RMS (2015):

The study is still considered to be supplementary.
Some details on the study design (e.g. inhalable particle content, sex of mice) are missing.
Under the conditions of the study and based on the information given in the report, the RD_{50} values (1 h-exposure, nose-only) values after exposure to cyfluthrin (aerosol, PEG) in rats and mice were 47 and 51 mg/m^3 air, respectively. The subacute exposure (6 h/day, 5 days/week/ 4 consecutive weeks) did not affect the responsiveness or magnitudes of changes in breathing patterns. This indicates that the effects observed are non-cumulative. Furthermore, pyrethroid burden in the indoor environment from carpets seems to low.

B.6.8.2.7 Other routes

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.1 /07
Report:	[redacted] 1985, TOX9550254 Range finding test for acute toxicity to the dog. Report no.: 13726 (August 14, 1985); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compul-

sory.

Deviations: 1 dog per dose (male or female).

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered not acceptable.

(dates of exp. work: April - July 1985).

Only the part for parenteral administration is presented (oral administration is presented in the chapter evaluating acute oral toxicity).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 98.5 %) was intravenously administered to one male and one female beagle dog ([redacted]) in a dose of 3.6 and 5.1 mg/kg bw, respectively. The substance was applied in PEG 400 in a volume of 0.9 (male) and 1.3 mL/kg bw (female). The substance was injected into the vena jugularis.
Recording period: 0-14 days.
Body weight: Day 0, 7, 14.
Necropsy: As soon as possible after the dead of the female dog.

Results and discussions:

Intravenous LD_{50} approx. 5 mg/kg bw.
Onset of death: 40 min p.a. (female dog).
Clinical signs: Already during the application: Convulsions, uncontrolled movements with the extremities, laying on the side, impaired respiration, bloody foam around the muzzle, vocalisation due to pain.
Body weight: Loss of weight (the surviving dog).
Gross pathology: No special features.

Conclusion:

Beta-cyfluthrin formulated in PEG 400 was of high acute intravenous toxicity to dogs.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be supplementary. However, due to the critical points listed below the study is now considered to be no longer acceptable. It should be noted that materials and methods section as well as the results section were revised.
The acute i.v. toxicity was studied in beagle dogs. At the time the study was conducted, no suitable OECD-guideline was compulsory. The study is considered not acceptable.
It is criticisable that the whole experiment was performed with merely 2 dogs (1 per dose, different sex per dose). Each male and female dog received the test compound in PEG 400. The observation time after administration of the test compound covered 14 days. The female animal died during the experiment. Under the conditions of the study and based on the information given in the report, the i.v. LD_{50} value was below 5 mg/kg bw. Clinical signs (for example convulsions, impaired respiration and uncontrolled movements) were noticed. The NOAEL (based on clinical signs) is <3.6 mg/kg bw.

Data point: KCA 5.8 /02

Report: [redacted] 1987, TOX9550269
FCR 4545 - technical - Study of the acute intraperitoneal toxicity to rats (formulation in polyethylene glycol E 400).
Report no.: 16104 (October 13, 1987); [redacted]

[redacted]

Guideline(s): At the time the study was performed, no particular method was compulsory.

Deviations: The negative controls were not mentioned in the report (only in the dossier).

GLP: The test followed the OECD principles of GLP (declaration of testing facility).

Acceptability: The study is considered acceptable.

(dates of exp. work: March - April 1986).

Materials and methods:

Beta-cyfluthrin (batch no.: 16002/84, purity: 99.1 %) was administered intraperitoneally to Wistar rats (Bor:WISW (SPFCpb), source: [redacted]) in the following dosing schedule:
0-1-5-7.1-10-11.2-12.5-25 mg/kg bw, 5 male rats/dosage group,
0-1-10-15-16-18-20*-25-50 mg/kg bw, 5 female rats/dosage group (* 10 animals). The formulating agent was PEG 400, the administered volume 5 ml/kg bw.
Recording period: 0-14 days.
Body weight: Daily.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

For intraperitoneal LD_{50} in rats see table below.
Onset of death: 97 min to ca. 5 h p.a.
Clinical signs: Lethargy, digging and preening movements (females only), uncoordinated gait, splayed gait, salivation, difficult breathing (beginning at ca. 20 min for up to a maximum of ca. 3 days). No delayed effects.
Body weight: Body weight reduction in a few females in all dose groups, compensated at day 14.
Gross pathology: Animals dying intercurrently: Lung: distended; kidney: mottled (males only); liver: with lobular pattern.
Animals sacrificed at the end of observation: Liver: swollen, lobes adhered to each other and in some cases also adhered to diaphragm and mesentery.

Table B. 6.8-20: Intraperitoneal LD_{50} in rats

	Formulation agent	**NOAEL [mg/kg bw]#**	**LD_{50} [mg/kg bw]***
Male rats	PEG 400	1	8.5 (7.3-10)
Female rats	PEG 400	1	17 (15.3-20)

= Maximum dosage without clinical signs.
* = () Confidence interval (95%).

Conclusion:

The clinical signs were reversible. The gross pathological findings were dose related and regarded as the result of the local irritating effect of the test compound formulation in PEG 400.
Beta-cyfluthrin formulated in PEG 400 was of a high acute toxicity after intraperitoneal administration to rats.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results and discussion section were revised.
For i.p. acute toxicity studies no guideline was available. Under the conditions of the study and based on the information given in the report, i.p. LD_{50} values (test compound formulated in PEG) for male and female rats amounted to 8.5 and 17 mg/kg bw, respectively.
Clinical signs (e.g. lethargy, digging and preening movements and uncoordinated gait) were observed. Only the lowest dose applied did not lead to clinical signs.

Data point:	KCA 5.8 /03
Report:	[redacted] 1988, TOX9550270 FCR 4545, FCR 1272 - Comparative study of the acute intraperitoneal toxicity to mice (formulation in polyethylene glycol E 400). Report no.: 17423 (November 25, 1988); [redacted]
Guideline(s):	At the time the study was performed, no particular method was compulsory.
Deviations:	The negative controls were not mentioned in the report (only in the dossier).
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	The study is acceptable.

(dates of exp. work: August 1987 - October 1987).

Purpose of the study:
The study was done in order to determine whether a shift in the percentage distribution of enantiomers in the mixture of the active substances beta-cyfluthrin and cyfluthrin has an effect on the acute intraperitoneal toxicity to mice. The enantiomer content in the active substances was as follows:

Table B.6.8-21: Enantiomer content in the active substances

	beta-cyfluthrin	cyfluthrin
Enantiomer I	0.3 %	24.7 %
Enantiomer II	35.8 %	19.4 %
Enantiomer III	0.7 %	33.5 %
Enantiomer IV	63.2 %	22.4 %

Materials and methods:

To 5 male mice per dosage group (NMRI [SPFHan], source: [redacted]) beta-cyfluthrin and cyfluthrin were singly intraperitoneally administered in the following dosing schedule:
beta-cyfluthrin (batch no. 16001/87, purity: 98 %): 0-10-16-17-18-20-25-100 mg/kg bw,
cyfluthrin (batch no.: 233690489, purity: 95.5 %): 0-1-10-25-40-50-63-71-100 mg/kg bw.
The formulating agent was PEG 400, the administered volume 5 mL/kg bw.
Recording period: 0-14 days.
Body weight: Day 0, 7, 14.
Necropsy: All animals (survivors sacrificed by diethyl ether asphyxiation).
Statistical method: LD_{50} calculation according to the method of Rosiello et al., (1977) and Baird and Balster (1979) (based on the method of Bliss), modified by Pauluhn (1983).

Results and discussions:

Onset of death: beta-cyfluthrin: ca. 50 min to ca. 2 h p.a., cyfluthrin: ca. 30 min to ca. 1 day.
Clinical signs: With the exception of a spasmodic state (beta-cyfluthrin only), signs no significantly different after administration of beta-cyfluthrin or cyfluthrin (lethargy, uncoordinated gait, splayed gait, spastic gait, rolling over, convulsions, difficult breathing (beginning shortly p.a. for up to a maximum of 1-3 days).
No delayed effects.
Body weight: No effect.

Table B. 6.8-22: Intraperitoneal LD_{50} of beta-cyfluthrin and cyfluthrin in mice

	Formulation agent	NOAEL [mg/kg bw]#	LD_{50} [mg/kg bw]*
beta-cyfluthrin	PEG 400	<10	18 (17-21)
cyfluthrin	PEG 400	1	63 (49-82)

= Maximum dosage without clinical signs.
* = () Confidence interval (95 %).

Gross pathology:
Animals dying intercurrently - beta-cyfluthrin: lungs; slightly distended in some cases; liver, kidneys and (in some cases) spleen: somewhat pale; renal pelvis reddened
Animals dying intercurrently - cyfluthrin: lungs: mottled, dark red in some cases, distended; liver and kidneys: somewhat pale; liver: mottled, slight lobular pattern; spleen and kidneys: mottled.
Animals sacrificed at the end of observation – beta-cyfluthrin: 1 animal: dark red zones in lung surface; otherwise no substance-induced changes
Animals sacrificed at the end of observation – cyfluthrin: no substance-induced changes

Conclusion:

Compared to cyfluthrin, the higher content of enantiomers II and IV in beta-cyfluthrin resulted in a higher acute toxicity after intraperitoneal administration to mice.

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the results section was revised.
The acute toxicity study (no guideline available) was performed with mice.
Under the conditions of the study and based on the information given in the report, i.p. LD_{50} values (test compound formulated in PEG) for male mice was 18 or 63 mg/kg bw for beta-cyfluthrin or cyfluthrin, respectively.
Clinical signs (e.g. lethargy, uncoordinated gait, splayed gait, spastic gait,) were observed. Only the lowest dose of cyfluthrin applied did not lead to clinical signs. After beta-cyfluthrin administration clinical signs were observed also at the lowest dose applied.

Data point: KCA 5.8.2 /01

Report: [redacted] 1980, TOX9550282
Determination of the acute toxicity (LD_{50}) - FCR 1272; Diastereomeres. Letter.
Report no.: 99168 (September 03, 1980); [redacted]

Guideline(s): At the time the study was performed, no particular method was compulsory.

Deviations: Purity and batch not given.
Source of animals not given.
Body weight: Not determined.
Necropsy: Not performed.
Statistical method: Not given.
No confidence interval given.
Clinical signs: Not specified.
Onset of death: Not specified.

GLP: When the study was performed, GLP was not compulsory.

Acceptability: The study is considered not acceptable.

(dates of exp. work: not given).

The results in this letter provide supplementary information.

Materials and methods:

To mice (NMRI, source: not given) the diastereomers I, II, III and IV were singly intraperitoneally or orally administered in the following dosing schedule:

Table B. 6.8-23: Dosing schedule

Diast. (no. of animals)	Oral appl. (mg/kg bw)	Intrap. appl. (mg/kg bw)
I (5 or 10)	250-5000	50-500
II (10 or 20)	15-250	10-25
III (5 or 10)	250-5000	50-2500
IV (10)	250-5000	250-1000

The formulating agent was PEG 400, the administered volumes 5 or 10 mL/kg bw. Recording period: 0-14 days. Body weight: Not determined. Necropsy: Not performed. Statistical method: Not given.

Results and discussions:

Table B. 6.8-24: Acute toxicity of diastereomers I, II, III and IV

Diastereomers	Oral LD_{50} (mg/kg bw)	Intrap. LD_{50} (mg/kg bw)
I	>5000	99
II	31	17
III	>5000	>2500
IV	>5000	630

Clinical signs were observed at the lowest dose of each diastereomer.

Table B. 6.8-25: NOAEL[#] of diastereomers I, II, III and IV

Diastereomers	Oral (mg/kg bw)	Intrap. (mg/kg bw)
I	<250	<50
II	<15	<10
III	<250	<50
IV	<250	<250

= maximum dosage without clinical signs.

Conclusion:

Diastereomer II was the most toxic, for both oral and intraperitoneal application. For oral application it was not possible to differentiate between the three other diastereomers, since the LD_{50} values were higher than the maximum applicable dose of 5000 mg/kg bw.

Re-evaluation by the RMS (2015):

The study is now considered to be not acceptable. In the original monograph of beta-cyfluthrin from October, 1996 (ASB2010-10436) the study was considered to be supplementary. However, due to the critical points listed below the study is now considered to be no longer acceptable. It should be noted that the results section was revised.
To investigate the acute toxicity of the cyfluthrin diastereomers via oral or i.p. administration, fasted male mice were used.
The main reason for the decision against acceptability is the absence of information concerning purity and batches used. Furthermore, there is a strong lack of data (e.g. source of animals, body weight [development], specific clinical signs, onset of death, statistical method and confidence interval not given). Necropsy was not performed.
Under the conditions of the study and based on the information given in the report, the LD_{50} of the diastereomers were > 5000, 31, > 5000 and > 5000 mg/kg bw after oral administration for diastereomers I, II, III and IV, respectively. For intraperitoneally administered diastereomers the LD_{50} values were 99, 17, > 2500, 630 mg/kg bw for diastereomer I, II, III and IV, respectively.
Clinical signs (although not specified) were observed already at the lowest dose of each substance.

The studies summed up below were not (for this specific application) submitted by the applicant for renewal of approval. However, the studies are available to the RMS (e.g. from other applications).
The mean features of the studies of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are presented. Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

(a) i.p. treatment
The LD_{50} for cyfluthrin after i.p. administration in PEG 400 was 66 mg/kg bw in male and 104 mg/kg bw in female rat (Flucke and Thyssen, 1980, 8800, TOX9401853) or 34 mg/kg bw in male rat and 94 mg/kg bw in female rat (Heimann, 1982, 10931, TOX9401854). If cremophor/water was used as solvent for cyfluthrin, a LD_{50} of 20 mg/kg bw in male rat and 24 mg/kg bw in female rat (Heimann, 1982, 10931, TOX9401854) was determined.

(b) s.c. treatment
The s.c. LD_{50} of cyfluthrin in PEG 400 was determined to be >2500 mg/kg bw in male and female mice (Flucke and Thyssen, 1980, 8800, TOX9401853).

B.6.8.2.8 Sensory irritant potential

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Data point:	KCA 5.2.4 /02
Report:	[redacted] 1988, TOX9550263 FCR 4545 (c. n. cyfluthrin K+L, proposed) - Study for sensory irritant potential in the rat (RD50 determination). Report no.: 16762 (May 31, 1988), [redacted]

Guideline(s):	At the time the study was performed, no particular guideline method was compulsory. The method based on ASTM E 981-84 and methods described in the literature.
Deviations:	It remains unclear how NOAEC was defined under the conditions of the study.
GLP:	No. When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(Dates of exp. work: March - May 1988).

Materials and methods:

The sensory irritant potential after acute inhalation was tested in order to establish a maximum tolerated air concentration (not sensory irritant) based on general recommendations for this parameter.
Beta-cyfluthrin (batch no: 16001/87, purity 96.3 %) was given to 4 male rats/group (strain Bor: WISW [SPFCpb], source: [redacted]) in a dynamic inhalation apparatus for 45 min (head-nose only). The test substance was examined as an aerosol, dissolved in 50 % ethanol/ 50 % PEG 400. The nominal concentrations were 0 (air, vehicle) and 10-50-160-500-1000 mg/m³ air corresponding to analytical concentrations of 0.7-5.5-14.5-58.5-91.2 mg/m³ air.
Recording period: 0-7 days.
Body weight: Day 0, 1, 3, 7.
Necropsy: All animals (survivors sacrificed by Evipan).
The following lung function parameters were determined: Peak expiratory flow (PEF) [seconds]; tidal volume (TV) [mL]; respiration rate (RR); minute volume (MV) [mL]; inspiration time (IT) [seconds]; expiration time (ET) [seconds].
The concentration of the test substance in the atmosphere was determined analytically; the size distribution of aerosol particles was measured. For determination of the RD_{50} (Respiratory Decrease) the minimum for the smoothed measured respiratory rate was calculated. The relative frequency decrease was calculated as a function of concentration using the Least square method.
Statistical methods: Different methods for different parameters (Chi-square-test, Fisher-test, Variance analysis (ANOVA), Box's test, Tukey-Kramer-test, modified by Games and Howell).

Results and discussions:

Table B.6.8-26: Inhalative RD_{50} in rats

	Formulation agent	**NOAEC [mg/m³]#**	**RD_{50} [mg/m³]***
Male rats (45 min, aerosol)	ethanol/ PEG 400	approx. 0.3	38 (15-291)

= concerning sensory irritant potential, determined via extrapolation.
* = determined on basis of lung function test (respiratory rate); = () Confidence interval (95 %).

Analysis of test atmosphere: Inhalable particle content ca. 100 % (particles <5 µm), particle analysis not performed in vehicle control and 0.7mg/m³ air (analytically determined).
Clinical signs: From 14.5mg/m³ air (analytical) onward: Piloerection, decreased respiration, salivation (58.5 and 91.2 mg/m³ air (analytical) and for rats receiving 91.2 mg/m³ air (analytical) additionally nasal irritation, scratching in the area of the nose (0 to 24 h).
Body weight: No effect.
Gross pathology: Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.
Lung function test: No toxicologically relevant differences between the air-exposed and vehicle-exposed rats were reported. Changes in the lung function parameters RR, IT, ET occurred from 14.5 mg/m³ onward. RD_{50}: 38 mg/m³ air (analytical, confidence interval [95 %]: 15-291 mg/m³ air). The extrapolated NOAEC amounts to approx. 0.3 mg/m³ air.

Conclusion:

Beta-cyfluthrin possessed a relatively high sensory irritant potential. The extrapolated NOEL was 0.3 mg/m³ air (calculation based on the minimum value of respiration frequency decrease).

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results section were revised.
The purpose of the study was to investigate the sensory irritant potential of the test compound. It should be noted that for this type of evaluation no particular OECD-Guideline was available at the time the study was conducted.
Male rats were head-nose exposed for 45 min to an aerosol of the test compound (maximum analytical concentration was 91.2 mg/m³). A 1:1 mixture of 50 % ethanol and 50 % polyethylene glycol E 400 served as vehicle.
The RD_{50} value – concentration inducing a 50 % decrease in respiratory rate - was calculated after performing lung function tests.
Under the conditions of the study and based on the information given in the report, the RD_{50} value of the test compound is 38 mg/m³ air. Clinical signs (e.g. decreased respiration and piloerection) were reported from an analytical concentration of 14.5 mg/m³ air onward. It remains unclear how NOAEC was defined under the conditions of the study. Taken together, it can be concluded that the test compound possesses a relatively high sensory irritant potential if applied as aerosol.

Data point:	KCA 5.2.4 /03
Report:	[redacted] 1988, TOX9550262 FCR 4545 (c. n. cyfluthrin K+L, proposed) - Study for sensory irritant potential in the mouse (RD50 determination). Report no.: 16694 (May 09, 1988) [redacted]
Guideline(s):	At the time the study was performed, no particular guideline method was compulsory. The method based on ASTM E 981-84 and methods described in the literature.
Deviations:	It remains unclear how NOAEC was defined under the conditions of the study.
GLP:	No. When the study was performed, GLP was not compulsory.
Acceptability:	The study is acceptable.

(Dates of exp. work: January - February 1988).

Materials and methods:

The sensory irritant potential after acute inhalation was tested in order to establish a maximum tolerated air concentration (not sensory irritant) based on general recommendations for this parameter.
Beta-cyfluthrin (batch no: 16001/87, purity 98 %) was given to 4 male mice/group (strain OF4 [SPF pure-bred], source: Lab. animal breeder IFFACREDO, L'Arbresle, France) in a dynamic inhalation apparatus for 45 min (head-nose only). The test substance was examined as an aerosol, dissolved in 50 % ethanol/ 50 % PEG 400. The nominal concentrations were 0 (vehicle) and 10-50-100-200-350-600-1000 mg/m³ air corresponding to analytical concentrations of 1.7-5.0-10.6-22.8-36.9-49.7-93.8 mg/m³ air.
Recording period: 0-7 days.
Body weight: Day 0, 1, 3, 7.
Necropsy: All animals (survivors sacrificed by Evipan).
The following lung function parameters were determined: Peak expiratory flow (PEF) [seconds]; tidal volume (TV) [mL]; respiration rate (RR); minute volume (MV) [mL]; inspiration time (IT) [seconds]; expiration time (ET) [seconds].

The concentration of the test substance in the atmosphere was determined analytically; the size distribution of aerosol particles was measured. For determination of the RD_{50} (Respiratory Decrease) the minimum for the smoothed measured respiratory rate was calculated. The relative frequency decrease was calculated as a function of concentration using the Least Square method.
Statistical methods: Different methods for different parameters (Chi-square-test, Fisher-test, Variance analysis (ANOVA), Box's test, Tukey-Kramer-test, modified by Games and Howell).

Results and discussions:

Table B.6.8-27: Inhalative RD_{50} in mice

	Formulation agent	NOAEC [mg/m³]#	RD_{50} [mg/m³]*
Male mice (45 min, aerosol)	ethanol/ PEG 400	approx. 2	37 (20-115)

= concerning sensory irritant potential.
* = determined on basis of lung function test (respiratory rate); = () Confidence interval (95 %).

Analysis of test atmosphere: Inhalable particle content ca. 100 % (particles ≤5 µm), particle analysis not performed in vehicle control, 1.7, 5.0 and 22.8 mg/m³ air (analytical determined).
Clinical signs: From 10.6 mg/m³ air (analytical) onward: Dose dependent slight to strong decrease in respiration, at 93.8 mg/m³ air (analytical) reduced motility (durations of clinical signs up to 3 h).
Body weight: No effect.
Gross pathology: Animals sacrificed at the end of observation: No indications of substance-induced grossly apparent organ damage.
Lung function test: A lower RR occurred from 10.6 mg/m³ air (analytical) onward. Significant changes in the IT, ET and TV were reported only at 93.8 mg/m³ air (analytical). RD_{50}: 37 mg/m³ air (analytical, confidence interval (95 %): 20-115 mg/m³ air). The NOAEC is approx. 2 mg/m³ air.

Conclusion:

Beta-cyfluthrin possessed a relatively high sensory irritant potential. The NOEL was approx. 2 mg/m³ air (calculation based on the minimum value of respiration frequency decrease).

Re-evaluation by the RMS (2015):

The study is still considered to be acceptable. It should be noted that the materials and methods section as well as the results and discussion section were revised.
The focus of the study was set on the sensory irritant potential of the test compound. There was no particular OECD-Guideline for this type of evaluation available at the time the study was conducted.
Male mice were head-nose exposed for 45 min to an aerosol of the test compound up to a maximum analytical concentration of 93.8 mg/m³. A 1:1 mixture of 50 % ethanol and 50 % polyethylene glycol E 400 served as vehicle.
The RD_{50} value – concentration inducing a 50 % decrease in respiratory rate – was determined after performing lung function tests.
Under the conditions of the study and based on the information given in the report, the RD_{50} value of the test compound is 37 mg/m³ air. From an analytical concentration of 10.6 mg/m³ air onward, animals showed slower or decreased respiration. It remains unclear how NOAEC was defined under the conditions of the study. In summary, the test compound – if applied as aerosol – possesses a relatively high sensory irritant potential

Further studies available to RMS

The studies listed below addressing the sensory irritant potential of cyfluthrin were not submitted by the applicant for renewal. However, the studies are available to RMS (e.g. from other applications).

The mean features of the studies – if evaluated as acceptable or supplementary (exclusion criteria e.g. no purity given, strong deviations from study design or questionable reliability) – are presented in the following table. Each evaluation is based on the today's criteria. Nevertheless, the outcomes do not alter the overall evaluation derived from the other studies presented in the RAR.

Table B. 6.8-28: Summary of acute toxicity studies – cyfluthrin

Vehicle	Species	Sex	Result [mg/m³]	Comment	Reference
ethanol:PEG 400 (1:1), aerosol	mouse (45-min exposure, head/nose only)	male	RD_{50}~67	- 4 rats per concentration and sex (similar guidelines advise 5) - observation for 7 days	[redacted], 1988 (16713) TOX9401869
ethanol:PEG 400 (1:1), aerosol	rat (60 min-exposure, nose only)	male	RD_{50}~50	- observation for 7 days	[redacted], 1995 (24249) TOX9552072
PEG, aerosol	rat and mice (60 min-exposure, nose only)	male and female	RD_{50} (rat) = 47 RD_{50} (mice) = 51	- publication concerning pyrethroids /indoor use - inhalable particle content unclear	[redacted], 1996 (MO-01-001783) TOX2001-880[1]

[1] The study is part of the submitted dossier and decribed in section B.1.1.2.6.

For beta-cyfluthrin no further studies were available.

B.6.8.3 Endocrine disrupting properties

No specific data were submitted under this headline.

B.6.9 Medical data and information

Human/Medical data were evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436). In the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599), a human volunteer study (Ruddy et al, 1998, TOX2001-879) was evaluated. In the study, 1-h inhalation exposure to approx. 0.1 mg cyfluthrin/m^3 air appeared to be in the range of an irritant threshold concentration for humans. A proposal for classification and labelling was not made for Annex I inclusion, but in the present renewal process.
A literature research taking the last 10 years into consideration was made and the results are integrated together with the data for the renewal process in the respective chapter.

B.6.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Flucke, 1979, TOX9401953: Memorandum - Irritant Effects after Work with FCR 1272 - Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, August 10, 1979.
Subject: Memorandum concerning laboratory personnel who had been subject to irritant effects after working with cyfluthrin and had summarised their own experience.

Miksche, 1979, TOX9401954: Symptoms of irritation when working with FCR 1272 - Bayer AG, Institute for Toxicology, D-42096 Wuppertal, Germany, August 21, 1979.
Subject: Letter of medical department with a short case-description.

Faul, 1988, TOX9401959: Medical Data on Employees in Cyfluthrin Formulation - Bayer AG, Medical Department Dormagen, D-41539 Dormagen, Germany, March 28, 1988.
Subject: Statement concerning 10 factory employees who were subjected to yearly industrial medical examinations (summarised results of routine examinations in a plant of Bayer AG in Germany, 1986, 1987).

Kollert, 1988, TOX9401958: Medical Data on Workers employed in Cyfluthrin Production and Formulation / Reregistration of the Active Ingredient in Brazil - Bayer AG, Medical Department Elberfeld, D-42096 Wuppertal, Germany, August 21, 1979.
Subject: Summarising letter concerning yearly medical supervision of cyfluthrin producing employees in a plant of Bayer AG in Germany from 1982 to 1988.

As summarised in the above mentioned documents, skin and eye symptoms have been observed in connection with the handling of cyfluthrin. The observations relate to people who have handled the active substance (synthesis laboratory, manufacturing plant, formulation plant, toxicological laboratory). The observations included skin reactions such as pruritus, tautness and reddening of the facial skin, partial facial paraesthesia, signs of irritation in the oro-pharyngeal cavity and the eyes. After onset of the irritation signs, an elevated sensitivity, particularly to touch stimuli, was observed. The effects were reversible within a few hours. (Flucke, 1979; TOX9401953, Miksche, 1979, TOX9401954; Faul, 1984, TOX9401959).
Only one of 20 employees who were producing cyfluthrin in 1982 complained digestive symptoms, which he attributed to working with the agent (Kollert, 1988, TOX9401958).
No health problems or changes in well-being were mentioned in connection with handling of cyfluthrin when the work rules were observed (Faul, 1988, TOX9401959, Kollert, 1988, TOX9401958).
Conclusions were drawn that by precautionary measures such as the wearing of protective clothing and avoidance of direct and indirect contamination of the relevant skin areas and the eyes, effects of

cyfluthrin can be prevented (Flucke, 1979, TOX9401953; Miksche, 1979, TOX9401954; Faul, 1984, TOX9401957; 1988, TOX9401959).
As cited in literature (Vijverberg and Van Den Bercken, 1982, TOX9401956; Narahashi, 1989, TOX9401960), the above described symptoms are apparently a result of the stimulation of the nervous system according to a specific interaction between the pyrethroids and the sodium channels of the nerve membranes. Series of nerve impulses are induced as a result of a change in the sodium permeability of the membranes (repetitive effect). The sensory organs and nerve-endings react particularly sensitively, and it appears that the dermal sensations experienced by the people after exposure to α-cyano-3-phenoxybenzyl alcohol pyrethroids are triggered by series of impulses in the sensory nerve endings (Aldridge, 1990, TOX9401961; Vijverberg and Van Den Bercken, 1990, TOX9401962).
This result of the primary effect of pyrethroids on the sodium channels is completely reversible within a matter of hours to a day. Neurological investigations in exposed people have not revealed any functional anomalies of the nerves of the arms and legs (Le Quesne et al., 1980, TOX9401955).

Further studies not submitted by the applicant but available to RMS (e.g. for other applications):

Faul, 1995, TOX2005-1679: Occupational medical experience: Individual cases of prickling irritation of the skin, with or without visible skin irritancy, mainly in facial, neck regions following direct skin contact (generally unnoticed), spontaneous reversal within 24-36 hours. No other observations of impairments to health or permanent effects, no changes in laboratory parameters following general occupational medical examinations.

Faul, 1984, TOX9401957: Letter, Bayer Medical Department - Bayer AG, Medical Department Dormagen, D-41539 Dormagen, Germany, July 2, 1984: Flubendizime (SLF 0312); Triazoxide (SAS 9244), Cyfluthrin (FCR 1272): Subject: Statement relating to occupational medical experience obtained over two years in a plant of Bayer AG in Germany.
Far greater training, more sophisticated plant technology and stricter protective measures are needed when handling the active ingredient cyfluthrin as a dust formulation. Just slight dust contact with the skin or mucosa of the eye, initially unnoticed, results in an unpleasant irritation and burning sensation at the point of contact within a few hours (first signs generally occur after showering, which has been made obligatory by the company). Characteristic of the milder cases is the inability to make an objective finding, e.g. irritation taking the form of redness. Following symptomatic treatment, subjective complaints, and in more severe cases irritation, abate within a few hours to within max. two days without lesions occurring. Several cases of this kind have been observed amongst employees at the plant. Parts mainly affected were the facial skin and eyes, contact occurring when opening part of the plant during cleaning at production changeover.

Krauthausen, E., 1995, ASB2009-987: FCR 1272: Occupational medical experience: Skin irritation can occur where handled incorrectly without protective clothing, lasts 15-20 h (reversible).

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

Data point:	CA 5.9 /02
Report:	Steffens, 2014, ASB2014-7889 Occupational medical experiences with beta-cyfluthrin Company: Bayer CropScience AG Report No: - Edition No.: M-476492-01-1
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	No; not published
Acceptability: (Date: 2014-01-29)	Acceptable.

Occupational medical surveillance of workers exposed to beta-cyfluthrin performed since 9 years on a routine basis did not reveal any unwanted effects in the workers. Examination intervals are quarterly for workers in plant, otherwise annually. The examinations included laboratory parameters (i.e. liver enzymes, bilirubin, urea, creatinin, and lipid profile) and technical examinations (i.e. Chest X-ray, ECG, vision testing, spirometry). During the production period since 2005 two accidents with beta-cyfluthrin itself occurred in the workers, both being irritation of face and eyes respectively, which both resolved very quickly. This effect is well known for pyrethroids. No further consultations of the Medical Department due to work or contact with beta-cyfluthrin were required.

Conclusion:

Acceptable, based on the information given in the report.

B.6.9.2 Data collected on humans

Studies evaluated in the addendum 1 to the monograph of beta-cyfluthrin (2002, ASB2014-9599):

Data point:	KCA 5.8
Report:	Ruddy K. et al, 1998, TOX2001-879 Safety and Tolerability Study of FCR 1272 0.04 AE in Healthy Volunteers, ICR Project No. 011337, Report-No.: 11590, unpublished Inveresk Clinical Research, Research Park Riccarton, Edinburgh, U.K. (Experimental work from 16 July – 23 September 1996)
Guideline(s):	The study was conducted in accordance with the provisions of the Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983) and Hong Kong (1989) revisions. The study was approved by an independent ethics review committee of Inveresk Research
Deviations:	None
GLP:	The test followed the OECD principles of GLP (declaration of testing facility).
Acceptability:	Acceptable

(Dates of experimental work: July 1996 to September 1996).

Materials and methods:

Test material: Insecticide spray-can aerosol "FCR 1272 0.04 AE" (contains active ingredients 0.044 % (w/w) cyfluthrin and 0.22 % (w/w) piperonyl butoxide).
Test subjects: 10 healthy male volunteers aged 21–37 years.
The study was designed as an open study. At the outset of the study 5 subjects were to be exposed to different concentrations of cyfluthrin for up to 1 h dependent upon tolerability, 4 h apart on the same day. The defined concentrations were ≤0.1 mg cyfluthrin/m^3 air and 0.5-0.8 mg cyfluthrin/m^3 air respectively. The initial exposure concentration was not tolerated and the higher exposure concentration was then cancelled. Laboratory analysis revealed that the initial actual concentration of the test substance had exceeded the defined concentration. The protocol was then amended to allow a further 5 subjects, at a later date, to be exposed to a lower concentration of ≤0.075 mg cyfluthrin/m^3 air for up to 1 h dependent upon tolerability. On this occasion, to alleviate anxiety, the subjects were exposed to an atmosphere of placebo spray-can aerosol before the test substance. The safety and tolerability of cyfluthrin 0.04 AE was assessed by the measurement of vital signs, measurements of heart rate and blood pressure, clinical laboratory tests (haematology, clinical chemistry and urinalysis), examination of mucous membranes and reporting of adverse events. Plasma and urine sampling were performed for

detection of metabolite levels for Group 1. Urine sampling only was performed for Group 2.

Results and discussions:

There were no clinically significant or drug related abnormalities in vital signs, ECGs or clinical laboratory tests after either exposure session.
For the first exposure session the corrected initial actual concentration for the subjects was ca. 0.2 mg cyfluthrin/m^3 air. The corrected initial actual concentration for one subject was ca. 0.09 mg cyfluthrin/m^3 air. For the second exposure session the corrected initial actual concentration for the subjects was ca. 0.1 mg cyfluthrin/m^3.
Only 2 of the subjects in Group l tolerated the first exposure session for the defined period of l h. Four of the subjects experienced subjective adverse events (symptoms) which were considered to be "definitely" related to the test substance. The adverse events reflected irritation of the mucous membranes of the nose (4 instances), upper respiratory tract (coughing, 2 instances), throat and eyes (single instances). These adverse events were all mild or moderate in severity and resolved within l h without treatment.
Three subjects had no symptoms and one subject – who was exposed to an initial concentration of ca. 0.09 cyfluthrin/m^3 – had mild hyperaemia of the nasal mucosa on examination of mucous membranes following exposure.
All subjects in Group 2 tolerated the 20 min exposure to the placebo spray-can aerosol on the evening before exposure to the test substance and no adverse events were reported. This exposure session was designed to alleviate anxiety which may have been a contributing factor in certain subjects leaving the atmosphere early during the first exposure session.
The subjects all tolerated the second exposure session for the defined period of l h. Four of the subjects experienced subjective adverse events which were considered to be "definitely" related to the test substance. The adverse events reflected irritation of the mucous membranes of the nose (3 instances) and throat (2 instances). They were mild in severity and resolved within 1 h without treatment. A single subject had mild hyperaemia of the nasal mucosa on examination of the mucous membranes following exposure.
The subjective adverse events were all expected side effects of the test substance with reference to preclinical studies and observations in agrochemical workers and reflected irritation of the mucous membranes of the nose, throat, upper respiratory tract and eyes in order of frequency. They were all self-limiting and resolved within minutes after cessation of exposure. The objective evidence of hyperaemia of the nasal mucosa was very marginal and transient resolving within l h. There was no evidence of changes in the mucous membranes of the eyes, mouth or throat.
There were no clinically significant or drug related abnormalities in vital signs, ECG's or clinical laboratory tests after either exposure session.
It is evident that the test substance was tolerated better by the Group 2 subjects who were exposed to a corrected initial actual concentration of ca. 0.1 mg cyfluthrin/m^3 than the Group l subjects who – with the exception of one subject – were exposed to a corrected initial actual concentration of ca. 0.2 mg cyfluthrin/m^3 air.

Conclusion:

In conclusion an initial actual concentration of ca. 0.1 mg cyfluthrin/m^3 air appears to be in the range of an irritant threshold concentration for humans since only 2 (of 5) subjects showed transient signs of irritation of the mucous membranes and symptoms experienced were transient and self-limiting. Slightly higher concentrations caused similar effects of greater intensity in all subjects.

Re-evaluation by the RMS (2015):

Under the conditions of the study and based on the information given in the report, an initial actual concentration of ~0.1 mg cyfluthrin/m^3air seems to be in the concentration range associated with a threshold for irritant concentrations.
The study is considered acceptable under the conditions of the study and based on the information given in the report. Also in the addendum 1 to the monograph (2002, ASB2014-9599), the study was

considered acceptable.
No proposal for classification and labelling was made in the addendum 1 (2002, ASB2014-9599). Based on the results obtained in this study and on the information from the teratogenicity studies with inhalational exposure in rats ([redacted], 1988, TOX9401910, [redacted], 1993, TOX9401829) were respiratory disturbances and bradypnoea due to irritative aerosol concentrations of cyfluthrin occurred, we propose to classify beta-cyfluthrin / cyfluthrin for irritating properties as follows:
Classification and labelling for respiratory irritation according to Regulation (EC) No 1272/2008 (GHS):
Specific target organ tox.-single exp., cat. 3: May cause respiratory irritation

The applicant disagreed with this proposal and has requested an external consultant to review the existing data package. However, the proposal for STOT-SE cat. 3 '*May cause respiratory irritation'* is based on a human volunteer study, medical data (irritation of respiratory mucous membranes with coughing and sneezing after inhalative exposure to cyfluthrin in workers) and inhalational teratogenicity studies in rats. Due to the irritating properties of the test substance at different dose levels a reflex bradypnoea and respiratory disturbances occurred in the dams. In order to make the user aware of the need for protection, the designation of STOT-SE 3 H335 '*May cause respiratory irritation'* according to Regulation (EC) No 1272/2008 is proposed by the RMS.

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

No poisoning cases have been reported in literature for beta-cyfluthrin.
Product stewardship in BCS collected between 27 and 47 human cases per year from 2011 to 2013. While many calls were for information only or referred to definitely unrelated symptoms (e.g. infections), thus unrelated to beta-cyfluthrin, the only symptoms reported from airborne exposures were skin irritation and/or "Cold Burn", the paresthesias typical for skin contact to α-cyanopyrethroids, and airway irritation, in some cases provoking asthma-like reactions. These too, are well known for pyrethroids. Few allergic reactions were described, but the correlation to beta-cyfluthrin could not be established. Formulation ingredients and/or other substances the individuals were exposed to were more likely causes. No allergies have been observed in production. No systemic poisoning cases definitely related to beta-cyfluthrin have been reported, not even in a single case of a suicidal attempt.

B.6.9.3 Direct observations

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

B.6.9.3.1 Experiences with other pyrethroids

There are no documented cases of cyfluthrin intoxication. However, cases of experience with other α-cyano-pyrethroids have been reported.
A report from China describes a series of 573 cases of intoxication with α-cyano-pyrethroids (deltamethrin, fenvalerate and cypermethrin). On occupational exposure (229 cases), the initial symptoms were burning, itching or tingling sensations of the face. The skin symptoms could appear early after several minutes of spraying, followed by systemic symptoms (i.e. dizziness, headache, nausea, anorexia, fatigue) commencing between 4 and 48 hours after exposure.
The principal signs after ingestion (a frequent route of exposure in the 344 cases of accidental intoxication) were of gastrointestinal nature (abdominal pain, nausea, vomiting, onset within 10 min to 1 h after exposure). Skin symptoms were not significant in patients of ingestion poisoning.
The more serious cases developed coarse muscular fasciculation's in large muscles of extremities. Clouding of consciousness and convulsions (lasting between 30 sec and 2 min and occurring 10-30 times per day) were recorded in a few cases. Treatment was of a symptomatic and supportive nature (gastric lavage, atropine for salivation and pulmonary oedema, diazepam, baclofen, phenobarbital,

chlorpromazine, phenytoin). The vast majority of patients recovered within 1 to 6 days, though the hospitalisation period of some seriously affected patients with convulsions was longer, the longest being 55 days. Fifteen cases were followed up and no long-standing or residual symptoms were found. In total, 7 cases (2 x occupational exposure to deltamethrin, 2 x ingestion of fenvalerate, 1 x pulmonary oedema, 1 x mistaken diagnosis, 1 x erroneous treatment) had a fatal outcome (He et al, 1989, TOX9401963).

B.6.9.3.2 Observation on exposure of the general population

There is no information on the exposure of the general population to cyfluthrin. Exposure may occur through residues of cyfluthrin which may be present in crops and during the application of insecticidal household and pet products that contain cyfluthrin.

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

No poisoning cases have been reported in literature for beta-cyfluthrin.

Literature search for the Renewal Assessment Report (RAR):

Data point:	KIIA 5.10
Report:	Das et al. (2006) ASB2015-929 Worker illness related to ground application of pesticide - Kern County, California, 2005. Morb Mortal Wkly Rep 55(17):486-488
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Acceptable

Abstract:
The Occupational Health Branch (OHB) of the California Department of Health Services (CDHS) conducts surveillance of work-related pesticide illness with support from the National Institute for Occupational Safety and Health (NIOSH) and the U.S. Environmental Protection Agency (EPA). On May 12, 2005, CDHS received a report from the California Department of Pesticide Regulation (CDPR) of a suspected pesticide incident in Kern County involving 27 farmworkers (age range: 21-61 years; median: 32.5 years) and six emergency responders (age range: 28-51 years; median: 33.5 years). CDHS investigated this incident by conducting a site visit; reviewing medical and meteorologic records; and interviewing affected workers, pesticide applicators, and the farmworker employer. Findings indicated that workers became ill from drift of a pyrethroid pesticide (cyfluthrin) that was being applied in a neighbouring field. Pyrethroid pesticide applicators should always operate in a manner that ensures workers are not exposed.
On May 12 at 7:00 a.m., a commercial pesticide application team was spraying in a citrus orchard to control thrip, a small insect that feeds on oranges. The pesticide solution contained 32 ounces of cyfluthrin (pyrethroid, EPA toxicity category I), 84 ounces of spinosad (EPA toxicity category III), 18.5 gallons of petroleum oil (EPA toxicity category III), and 1,800 gallons of water. The pesticide was sprayed from three enclosed ground rig applicator tractors that travelled up and down rows and turned around on a dirt road that borders the field. In a neighbouring grape vineyard southeast of the pesticide application, 27 farmworkers (23 female) were suckering (i.e., pruning unwanted shoots), lifting, and tying grape vines. Although employers are required by CDPR to notify their workers when they are within a quarter mile of cyfluthrin application, notification of farmworkers in the neighbouring vineyard was not required because they worked for a different employer.
The farmworkers continued to work, and spraying resumed approximately 20 minutes later. Shortly thereafter, some of the workers noticed a chemical odour, began feeling ill, and stopped working.

Twenty-three workers (all female) were decontaminated on site by the hazardous material team (HAZMAT). They were then transported by ambulances to local hospitals. Four other workers (all male), who had been lifting grape vines in a location further from the spraying, were identified later that day and transported by their supervisor to medical care the following day as a precaution. After evaluation in emergency departments, all 27 farmworkers were discharged home.
Symptoms most commonly reported by the 27 farmworkers were headache (96 %), nausea (89 %), eye irritation (70 %), muscle weakness (70 %), anxiety (67 %), and shortness of breath (64 %). Illness severity was classified according to a severity index for acute pesticide-related illness. Illness severity was moderate in five (19 %), low in 20 (74 %), and not applicable (i.e., less than two symptoms) in two (7 %) farmworkers. Because of the known toxicity of the different substances applied, these effects were attributed primarily to cyfluthrin. Illness symptoms were not reported by the applicators, who were wearing appropriate protective equipment. Foliage samples obtained by CDPR from the citrus orchard southeast of the pesticide spraying indicated cyfluthrin levels of 1.14 ppm. Neither foliage samples obtained from the grape vineyard nor clothing samples obtained from the farmworkers had measurable levels of cyfluthrin. CDHS is conducting follow-up with these workers to assess any potential persistent effects associated with acute cyfluthrin exposure.
Six emergency responders (four male) responded to the incident and were evaluated in emergency departments. Health effects were reported by four of six emergency responders and included respiratory (four), skin (three), and eye (two) symptoms. The illness severity rating was low in four of six emergency responders and, in two others, was not applicable.

Conclusion:

The incident described in this report highlights two potential occupational hazards in agriculture: pyrethroid toxicity and pesticide drift.

Data point:	KIIA 5.10
Report:	Miller (2014) ASB2015-928 Case Report: Human intravenous injection of beta-cyfluthrin with minimal toxic effects. American Journal of Emergency Medicine 32 (2014) 113e1-113e2
Guideline(s):	Not applicable
Deviations:	Not applicable
GLP:	Not applicable
Acceptability:	Supplementary

Abstract:
The American Journal of Emergency Medicine (Miller, 2014) reported that a 28-year-old man presented to the emergency department 20 minutes after injecting 20 ml of an insecticide containing 0.05 % beta-cyfluthrin. The cause for the injection remained unknown. The man showed sinus tachycardia as the only symptom and was treated with an intravenous fluid bolus of 2000 ml. After 3 hours he fully recovered.

Conclusion:

This patient experienced a benign clinical course and he fully recovered. Vigilance for pyrethroid toxic effects such as seizures, severe tremors, and choreoathetosis is paramount.

B.6.9.4 Epidemiological studies

Studies submitted with the dossier for the Renewal Assessment Report (RAR):
No data are available for beta-cyfluthrin.

An overview of pyrethroid (not specifically beta-cyfluthrin) toxicity has been given in Toxicol Rev, 24(2005), 93-106: 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.

B.6.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

Skin reactions such as pruritus, tautness and reddening of the facial skin, partial facial paraesthesia and signs of irritation in the oro-pharyngeal cavity or coughing, especially when concomitant with an elevated sensitivity, particularly to touch stimuli, may be signs of dermal contact with or inhalative exposure to cyfluthrin. Following inhalative exposure irritation of respiratory mucous membranes (coughing, sneezing) may occur.
Poisoning signs after oral ingestion of cyfluthrin are not known (see B.5.9.6 Expected effects of poisoning).

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

In cases of contact to pyrethroids the first sign of exposure is a specific paraesthesia/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 general poisoning.
The irritation can occur both on the skin and on the mucous membranes of the airways. In the latter case in sensitive 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:

Table B.6.9-1: Signs and symptoms 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	

B.6.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

An effective antidote for cyfluthrin or for other α-cyano-pyrethroid insecticides is not known. However, studies in rats (see section B.5.8.4 Antidote studies) indicate that the central nervous effects can be ameliorated by muscle relaxants: tetrazepam proved able to suppress toxic signs, delay the onset of death and increase the LD_{50}-value (Heimann, K.G., 1983, TOX9401941).

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

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 not very soluble in plain water
  Note: Warm water may increase the subjective severity of the irritation/paraesthesia, which is not a sign of systemic poisoning.
- Flushing of the eyes with lukewarm water for 15 minutes, apply soothing eyedrops, if needed anesthetizing eyedrops
- Induction of vomiting should only be considered if a significant amount has been swallowed (more than a mouthful), if the ingestion was less than one hour ago, and if the patient is fully conscious. Induced vomiting can remove maximum 50 % of the ingested substance.
- Note: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!

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/paraesthesia, 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.

Contraindications:
Adrenergic compounds (except for CRP) and high dose atropine. Pyrethroid poisoning should not be confused with carbamate or organophosphate poisoning. 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.

B.6.9.7 Expected effects of poisoning

Studies evaluated in the original monograph of beta-cyfluthrin by the RMS in October, 1996 (ASB2010-10436):

The effects of cyfluthrin poisoning are not known. However, one may expect signs similar to those

described from other α-pyrethroid compounds (see B.5.9.2 Direct observations).

Note RMS (2015): Meanwhile new information is available (see Direct observation, Miller, 2014, ASB2015-928).

Studies submitted with the dossier for the Renewal Assessment Report (RAR):

First Aid:
No late effects of pyrethroid poisoning have been described in the scientific literature. Recovery is spontaneous and without sequelae.

B.6.10 References relied on

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA Section 5 /02	Anon.	1987	Pesticide residues in food - 1987 - Cyfluthrin Journal:FAO Plant Production and Protection Paper, Year:1986, Report No.: M-460340-01-1, Edition Number: M-460340-01-1 GLP/GEP: n.a., published TOX9401964	Y	N		
		2000	Pesticide residues - Guidelines for the preparation of toxicological working papers for the WHO Core Assessment Group of the Joint Meeting on Pesticide Residues Geneva, December 2000; Appendix I: Approximate relation of parts per million in the diet to mg/kg bw per day and unit relationships http://www.who.int/foodsafety/chem/jmpr/guidelines/en/ ASB2013-4646				
	Germany	1996	beta-cyfluthrin (Monograph) GLP: N, published ASB2010-10436	N	N		LIT
	Germany	2002	Beta-Cyfluthrin: Draft Assessment Report, Addendum 1 (B.2, B.5, B.7-B.9) GLP: N, published ASB2014-9599	N	N		LIT

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8	[redacted]	1991	FCR 1272: Study for acute oral toxicity in rats Report No.: 19852 ! T 3034510 ! MO-01-002911 GLP: Yes Published: No TOX9401863 BVL-3094315	Y	N		Beta-Cyfluthrin Task Force
KCA 5.4	Brusick, D. J.	1982	Evaluation of FCR 1272 in the reverse mutation induction assay with saccharomyces cerevisiae strains S138 and S211 (revised September 1982) Report No.: 5.4 /07 ! Bayer R2248 ! T 1004269 ! MO-01-003128 GLP: No Published: No TOX9401896 BVL-3094340	N	N		Beta-Cyfluthrin Task Force
KCA 5.4	Brusick, D. J.	1982	Evaluation of FCR 1272 in the induced mitotic crossing over, reverse mutation and gene conversion assay in saccharomyces cerevisiae strain D7 (revised September 1982) Report No.: 5.4 /08 ! Bayer R2249 ! 20998 ! T 8004536 ! MO-01-003126 GLP: No Published: No TOX9401897 BVL-9034341	N	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4	Curren, R. D.	1985	FCR 1272: Unscheduled DNA synthesis in rat primary hepatocytes Report No.: 701 ! T4023.380 ! MO-01-003009 GLP: Yes Published: No TOX9401900 BVL-3094339	N	N		Beta-Cyfluthrin Task Force
KCA 5.2	[redacted]	1984	FCR 1272 (Cyfluthrin) & NAK 1654 (Fenfluthrin) - Untersuchungen zur Kombinationstoxizität Report No.: 12572 ! T 0008732 GLP: Open Published: No Z17076 BVL-3138621	Y	N		BAY
KCA 5.2	[redacted]	1985	FCR 4545 techn.: Untersuchungen zur akuten oralen Toxizität am Huhn (Gallus domesticus) Report No.: 13689 ! T1019902 GLP: No Published: No TOX9402234 BVL-1670302	Y	N		BAY
KCA 5.2.1	[redacted]	1980	FCR 1272: Acute toxicity studies Report No.: 8800 ! MO-01-002988 GLP: No Published: No TOX9401853 BVL-3094316	Y	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.2	[redacted]	1980	FCR 1272: Acute toxicity studies Report No.: 8800 ! MO-01-002988 GLP: No Published: No TOX9401853 BVL-3094318	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.4	[redacted]	1980	FCR 1272: Acute toxicity studies Report No.: 8800 ! MO-01-002988 GLP: No Published: No TOX9401853 BVL-3094328	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.5	[redacted]	1980	FCR 1272: Acute toxicity studies Report No.: 8800 ! MO-01-002988 GLP: No Published: No TOX9401853 BVL-3094330	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.6	[redacted]	1980	FCR 1272: Acute toxicity studies Report No.: 8800 ! MO-01-002988 GLP: No Published: No TOX9401853 BVL-3094333	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.1	[redacted]	1987	FCR 1272 (c.n. cyfluthrin): Study for acute oral toxicity to rats (formulation acetone and peanut oil) Report No.: 15847 ! MO-01-002713 ! T 1020955 GLP: Yes Published: No TOX9401862 BVL-3094314	Y	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4	Herbold, B.	1980	FCR 1272: Salmonella/microsome test for detection of point-mutagenic effects Report No.: 9273 ! FCR 1272/033 ! MO-01-003025 ! M-037526-03-1 GLP: No Published: No TOX9401890 BVL-3904346	N	N		Beta-Cyfluthrin Task Force
KCA 5.4	[redacted]	1980	FCR 1272: Micronucleus test on mouse to evaluate FCR 1272 for mutagenic potential Report No.: 9435 ! FCR 1272/047 ! MO-01-002459 GLP: No Published: No TOX9401891 BVL-3094337	Y	N		Beta-Cyfluthrin Task Force
KCA 5.4	Herbold, B.	1981	FCR 1272 (Cyfluthrin, Baythroid - active ingredient): Pol A1 test on E. coli to evaluate effects for DNA damage Report No.: 10450 ! T 8004220 ! MO-01-002540 GLP: No Published: No TOX9401893 BVL-3094345	N	N		Beta-Cyfluthrin Task Force
KCA 5.8	Herbold, B.	2002	FCR 1272-Phenoxyethylester: Salmonella/microsome test-Plate incorporation and preincubation method Report No.: PH-32175 ! T 2071194 GLP: Yes (1) Published: No TOX2002-1391 BVL-3094347	N	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.1	[redacted]	1981	FCR 1272 (Cyfluthrin): Acute oral toxicity to dogs Report No.: T 6010889 ! MO-01-005194 GLP: No Published: No TOX9401856 BVL-3094317	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.4	[redacted]	1982	FCR 1272: Eye and skin irritation study on rabbits Report No.: 233 ! MO-01-004694 GLP: No Published: No TOX9401872 BVL-3094328	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.5	[redacted]	1982	FCR 1272: Eye and skin irritation study on rabbits Report No.: 233 ! MO-01-004694 GLP: No Published: No TOX9401872 BVL-3094331	Y	N		Beta-Cyfluthrin Task Force
KCA 5.8	[redacted]	2002	FCR 1272-Phenoxyethylester: Study for acute oral toxicity in rats Report No.: PH-32041 ! T9071399 GLP: Yes Published: No TOX2002-1390 BVL-3094335	Y	N		Beta-Cyfluthrin Task Force
KCA 5.4	Nagane, M.; Hatanaka, J.; Iyatomi, A.	1982	FCR 1272: Mutagenicity test on bacterial system Report No.: 213 ! JAP213 ! MO-01-004657 GLP: No Published: No TOX9401894 BVL-3094342	N	N		Beta-Cyfluthrin Task Force
KCA 5.2.3	[redacted]	1984	Acute inhalation study of FCR 1272 on rats Report No.: 269 ! MO-01-004697 GLP: No Published: No TOX9401866 BVL-3094327	Y	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4	Ohta, T.; Moriya, M.	1982	FCR 1272: Microbial mutagenicity study Report No.: 5.4 /06 ! MO-01-004654 GLP: No Published: No TOX9401895 BVL-3094343	N	N		Beta-Cyfluthrin Task Force
KCA 5.8	[redacted]	1988	FCR 1272 (c.n. cyfluthrin, proposed): Study for sensory irritant potential in the mouse (RD50 determination) Report No.: 16713 ! T 5027583 ! MO-01-002830 GLP: No Published: No TOX9401869 BVL-3094322	Y	N		Beta-Cyfluthrin Task Force
KCA 5.8	[redacted]	1988	FCR 1272 (suggested common name: cyfluthrin): Study of the blood gases in rats Report No.: 16763 ! T 3027653 ! MO-01-002832 GLP: No Published: No TOX9401870 BVL-3094323	Y	N		Beta-Cyfluthrin Task Force
KCA 5.8	[redacted]	1988	FCR 1272 (c.n.: Cyfluthrin): Untersuchungen zur Konzentrationsabhängigkeit der Körpertemperatur (Hypothermie) an der Ratte (Exposition: 1 x 6 Stunden) Report No.: 17209 ! T 9030060 GLP: No Published: No Z14816 BVL-3138481 BVL-3094320 (engl. version)	Y	N		BAY

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.8	[redacted]	1987	FCR 1272 (generic name: cyfluthrin): Studies of acute inhalation toxicity in the mouse, in accordance with OECD guideline No. 403 Report No.: 17765 ! T 2030559 ! MO-01-002901 GLP: Yes Published: No TOX9401871 BVL-3094324	Y	N		Beta-Cyfluthrin Task Force
KCA 5.8	[redacted]	1995	FCR 1272 (c.n. Cyfluthrin): Study on the RD50-determination in rats Report No.: 24249 ! T 0059097 ! MO-01-003869 GLP: Open (1) Yes (4) Published: No TOX9552072 BVL-3094321	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2.3	[redacted]	1982	FCR 1272: Study for acute inhalation toxicology (effect of formulating agent on inhalation) Report No.: 10965 ! MO-00-013967 GLP: No Published: No TOX9401864 BVL-3094319	Y	N		Beta-Cyfluthrin Task Force
KCA 5.2	[redacted]	1985	Acute oral toxicity (LD_{50}) study with FCR 1272 (c.n. Cyfluthrin) vehicle: PEG 400 in the hen Report No.: 3622 ! 053638 ! T8021014 ! MO-01-003109 GLP: Yes, Published: No BVL-1753704 TOX9401861	Y	N		BAY

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2	[redacted]	1985	Acute oral toxicity (LD_{50}) study with FCR 1272 (c.n. Cyfluthrin) vehicle: cremophor (R) EL 2 % in distilled water in the hen Report No.: R 3621 ! 053640 ! T 7021013 ! MO-01-003110 GLP: Yes, Published: No BVL-1753703 TOX9401860	Y	N		BAY
KCA 5.4	Sasaki, Y. F. X.	1986	Cyfluthrin: *In vitro* cytogenetics test Report No.: MO-01-004656 GLP: Yes Published: No TOX9401901 BVL-3094344	N	N		Beta-Cyfluthrin Task Force
KCA 5.2.6	[redacted]	1994	FCR 1272 - Study for skin-sensitising effects in guinea pigs (Magnusson-Kligman Maximisation Test) Report No.:23060 ! T 4055473 ASB2007-2854 BVL-3094332	J	N		Beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.5	[redacted]	2000	Supplemental submission to Bayer report no. 107769 – incl. Technical grade Cyfluthrin: A combined chronic toxicity/oncogenicity testing study in the rat - Report No.:107769-1 ! 8384! 107769 GLP: Yes Published: No TOX2001-1766 BVL-1848373 (1 of 9; incl. Supplemental Report) BVL-1848374 (2 of 9) BVL-1745471 (3 of 9) BVL-1847618 (4 of 8) BVL-1848375 (5 of 9) BVL-1847581 (6 of 9) BVL-1847582 (7 of 9) BVL-1847568 (8 of 9) BVL-1847569 (9 of 9)	Y	N		BAY
KCA 5.7.1	Wolansky, M. J.; Gennings, C.; Crofton, K. M.	2005	Relative potencies for acute effects of Pyrethroids on motor function in rats Page: 271–277 TOXICOLOGICAL SCIENCES vol.89, 1 271–277 ASB2013-7265 BVL-3094356	J	N		LIT

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.4	Yang, L.	1985	FCR 1272: CHO/HGPRT mutation assay in the presence and absence of exogenous metabolic activation Report No.:694 ! T 4023.332 ! MO-01-003008 ! M-039037-01-1 GLP: No Published: No TOX9401899 BVL-3094338	N	N		beta-Cyfluthrin Task Force

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1 /04	[redacted]	1982	Comparitive study of rats on absorption of FCR after single oral administration in polyethylene glycol 400 or Cremophor EL/ water as formulation vehicle [redacted] Bayer CropScience, Report No.: PH 10715, Edition Number: M-038869-01-1 Date: 1982-03-10 GLP/GEP: no, unpublished RIP9400865	Y	N		Bayer CropScience
KCA 5.1.1 /01	[redacted]	1982	Comparative study of rats on absorption of FCR 1272 after single oral administration in polyethylene glycol 400 or cremophor EL/water as formulation vehicle [redacted] Bayer CropScience, Report No.: 10715, Edition Number: M-037401-01-1 EPA MRID No.: 00131517 Date: 1982-03-10 GLP/GEP: no, unpublished RIP9400865	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /02	[redacted]	1983	[U- ^{14}C] cyfluthrin ([U-^{14}C]) FCR 1272; fluorobenzene label) : Biokinetic part of the general metabolism studies in the rat [redacted] Bayer CropScience, Report No.: PH 11872 (F), Edition Number: M-038565-01-1 Date: 1983-06-09 GLP/GEP: no, unpublished **...also filed: KCA 5.1.2 /02** **...also filed: KCA 6.2 /01** **...also filed: KCA 6.4 /03** RIP9400867	Y	N		Bayer CropScience
KCA 5.1.1 /03	[redacted]	1983	Fluorophenyl-ul-^{14}C cyfluthrin (FCR 1272) biokinetic study on rats [redacted] Bayer CropScience, Report No.: PH 11575(F), Edition Number: M-136572-01-2 Date: 1983-02-18 GLP/GEP: no, unpublished **...also filed: KCA 6.2 /02** **...also filed: KCA 6.4 /04** RIP9400866	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /04	[redacted]	1983	<fluorobenzene-UL-^{14}C>FCR1272; <fluorobenzene-UL-^{14}C>cyfluthrin: metabolism part of the general metabolism studies in the rat [redacted] Bayer CropScience, Report No.: PF-2059, Edition Number: M-034022-01-1 Date: 1983-09-14 GLP/GEP: no, unpublished **...also filed: KCA 5.1.2 /03** **...also filed: KCA 6.2 /03** **...also filed: KCA 6.4 /05** RIP9400868	Y	N		Bayer CropScience
KCA 5.1.1 /05	[redacted]	1982	Biotransformation of [f-phenyl-UL-^{14}C] cyfluthrin; characterisation and preliminary identification of the metabolites [redacted] Bayer CropScience, Report No.: PF 1632, Edition Number: M-136574-01-2 Date: 1982-01-21 GLP/GEP: no, unpublished **...also filed: KCA 5.1.2 /05** **...also filed: KCA 6.2 /04** **...also filed: KCA 6.4 /06** RIP9400862	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /06	[redacted]	1983	Metabolism of Baythtroid in a dairy cow [redacted] Bayer CropScience, Report No.: MR86043, Edition Number: M-052654-01-1 Date: 1983-09-27 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /56** **...also filed: KCA 4.2 /11** **...also filed: KCA 5.1 /05** **...also filed: KCA 6.2.3 /01** **...also filed: KCA 6.4.2 /02** RIP9400870	Y	N		Bayer CropScience
KCA 5.1.1 /08	[redacted]	1987	Biotransformation of Cyfluthrin in the chicken after oral adminstration of a high dose [redacted] Bayer CropScience, Report No.: 15849, Edition Number: M-038063-01-1 Date: 1987-06-24 GLP/GEP: no, unpublished **...also filed: KCA 4.2 /13** **...also filed: KCA 6.4.1 /02** TOX9401851	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /09	[redacted]	2013	Beta-cyfluthrin: Absorption, distribution, excretion of [fluorophenyl-UL-^{14}C] beta-cyfluthrin in male rats after single oral administration at one dose level [redacted] BCS-Irvita, Report No.: D59956, Edition Number: M-481053-01-1 Date: 2013-03-06 GLP/GEP: yes, unpublished BVL-2632937, BVL-2632937, ASB2014-7716	Y	Y	bridging from old cyfluthrin data to beta-cyfluthrin	BCS-Irvita
KCA 5.1.1 /10	[redacted]	2014	Beta-cyfluthrin: Absorption, distribution, excretion and metabolism of fluorophenyl-UL-^{14}C] beta-cyfluthrin, formulated in PEG400, in male rats after single oral administration at one dose level [redacted] BCS-Irvita, Report No.: D69261, Edition Number: M-481060-01-1 Date: 2014-03-18 GLP/GEP: yes, unpublished BVL-2632938, BVL-2632938, ASB2014-7717	Y	Y	Bridging experiment from the existing cyfluthrin rat ADME study to beta-cyfluthrin	BCS-Irvita

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.1 /11	[redacted]	2013	Beta-cyfluthrin: Absorption, distribution, excretion and metabolism of [cyclopropane-1-^{14}C] beta-cyfluthrin in male and female rats after single oral administration at two dose levels [redacted] BCS-Irvita, Report No.: D59945, Edition Number: M-481047-01-1 Date: 2013-11-26 GLP/GEP: yes, unpublished BVL-2632939, BVL-2632939, ASB2014-7718	Y	Y	to address second radiolabel	BCS-Irvita
KCA 5.1.2 /02	[redacted]	1983	[U- ^{14}C] cyfluthrin ([U-^{14}C]) FCR 1272; fluorobenzene label) : Biokinetic part of the general metabolism studies in the rat [redacted] Bayer CropScience, Report No.: PH 11872 (F), Edition Number: M-038565-01-1 Date: 1983-06-09 GLP/GEP: no, unpublished **...also filed: KCA 5.1.1 /02** **...also filed: KCA 6.2 /01** **...also filed: KCA 6.4 /03** RIP9400867	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.2 /03	[redacted]	1983	<fluorobenzene-UL-^{14}C>FCR1272; <fluorobenzene-UL-^{14}C>cyfluthrin: metabolism part of the general metabolism studies in the rat [redacted] Bayer CropScience, Report No.: PF-2059, Edition Number: M-034022-01-1 Date: 1983-09-14 GLP/GEP: no, unpublished **...also filed: KCA 5.1.1 /04** **...also filed: KCA 6.2 /03** **...also filed: KCA 6.4 /05** RIP9400868	Y	N		Bayer CropScience
KCA 5.1.2 /04	[redacted]	1981	Thiocyanate excretion in rats urine after intraperitoneal administration of FCR 1272 and Decamethrin in comparable doses and after exposure to defined FCR 1272 concentrations in the inhalation air [redacted] Bayer CropScience, Report No.: 10130, Edition Number: M-037234-01-1 EPA MRID No.: 00131516 Date: 1981-08-17 GLP/GEP: no, unpublished **...also filed: KCA 6.2 /05** **...also filed: KCA 6.4 /07** RIP9400855	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.1.2 /05	[redacted]	1982	Biotransformation of [f-phenyl-UL-^{14}C] cyfluthrin; characterisation and preliminary identification of the metabolites [redacted] Bayer CropScience, Report No.: PF 1632, Edition Number: M-136574-01-2 Date: 1982-01-21 GLP/GEP: no, unpublished **...also filed: KCA 5.1.1 /05** **...also filed: KCA 6.2 /04** **...also filed: KCA 6.4 /06** RIP9400862	Y	N		Bayer CropScience
KCA 5.1.2 /06	[redacted]	2014	Beta-Cyfluthrin: Comparative in-vitro metabolism of [fluoro-phenyl-UL-14 C] - Beta-cyfluthrin in rat and human liver microsomes [redacted] BCS-Irvita, Report No.: M-482993-01-1, Edition Number: M-482993-01-1 Date: 2014-01-14 GLP/GEP: yes, unpublished **...also filed: KCA 6.2 /07** BVL-2632940, BVL-2632940, ASB2014-7719	Y	Y	new data requirement under Reg. 1107/2009; data not submitted on EU Level	BCS-Irvita

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.1 /01	[redacted]	1987	FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in Polyethylene Glycol E 400) [redacted] Bayer CropScience, Report No.: 16182, Edition Number: M-065818-01-1 EPA MRID No.: 41244102 Date: 1987-11-05 GLP/GEP: yes, unpublished TOX9550258	Y	N		Bayer CropScience
KCA 5.2.1 /02	[redacted]	1987	FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in Xylene) [redacted] Bayer CropScience, Report No.: 16176, Edition Number: M-137062-01-1 EPA MRID No.: 41244101 Date: 1987-11-04 GLP/GEP: yes, unpublished TOX9550255	Y	N		Bayer CropScience
KCA 5.2.1 /03	[redacted]	1987	FCR 4545 technical - Study of the acute oral toxicity to rats (formulation in Acetone/Peanut Oil) [redacted] Bayer CropScience, Report No.: 16181, Edition Number: M-065814-01-1 EPA MRID No.: 41244104 Date: 1987-11-05 GLP/GEP: yes, unpublished TOX9550257	Y	N		Bayer CropScience

Annex point / reference number	Author(s)	Year	Title Source *(where different from company)* Company name, Report No., Date, GLP status *(where relevant)*, published or not	Vertebrate study Y/N	Data protection claimed Y/N	Justification if data protection is claimed	Owner
KCA 5.2.1 /06	[redacted]	1987	FCR 4545 technical. - Study of the acute oral toxicity to mice (formulation in polyethylene glycol E 400) [redacted] Bayer CropScience, Report No.: 16177, Edition Number: M-065392-01-1 EPA MRID No.: 41244103 Date: 1987-11-04 GLP/GEP: yes, unpublished TOX9550256	Y	N		Bayer CropScience
KCA 5.2.1 /08	[redacted]	1987	FCR 1261 - Study for acute oral toxicity to rats [redacted] Bayer CropScience, Report No.: 15419, Edition Number: M-042268-01-1 Date: 1987-01-14 GLP/GEP: no, unpublished TOX9401933	Y	N		Bayer CropScience
KCA 5.2.1 /09	[redacted]	1986	FCR 3191 = THS 2997 - Study for acute oral toxicity in rats [redacted] Bayer CropScience, Report No.: 14800, Edition Number: M-042141-01-1 EPA MRID No.: 41190203 Date: 1986-07-07 GLP/GEP: no, unpublished TOX9401930	Y	N		Bayer CropScience

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KCA 5.2.1 /10	[redacted]	1987	FCR 3145 = RAD 69/86 - Study for acute oral toxicity to rats [redacted] Bayer CropScience, Report No.: 15532, Edition Number: M-037961-01-1 Date: 1987-02-06 GLP/GEP: no, unpublished TOX9401934	Y	N		Bayer CropScience
KCA 5.2.1 /11	[redacted]	1986	FCR 2947 = THS 3010 - Study for acute oral toxicity to rats [redacted] Bayer CropScience, Report No.: 14799, Edition Number: M-042131-01-1 Date: 1986-07-07 GLP/GEP: no, unpublished TOX9401929	Y	N		Bayer CropScience
KCA 5.2.1 /12	[redacted]	1986	FCR 2728 = THS 3028 - Study for acute toxicity to rats [redacted] Bayer CropScience, Report No.: 15239, Edition Number: M-042156-01-1 Date: 1986-10-28 GLP/GEP: no, unpublished TOX9401931	Y	N		Bayer CropScience

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KCA 5.2.1 /13	[redacted]	1986	FCR 2978 = THS 3062 - Study for acute toxicity to rats [redacted] Bayer CropScience, Report No.: 15241, Edition Number: M-042161-01-1 Date: 1986-10-28 GLP/GEP: no, unpublished TOX9401932	Y	N		Bayer CropScience
KCA 5.2.1 /14	[redacted]	1996	Cyfluthrin: Concentration of the parent compound in blood plasma, brain and omental fat of rats following administration with the feed or by oral administration [redacted] Bayer CropScience, Report No.: MR-625/95, Edition Number: M-044715-01-1 Date: 1996-03-15 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /79** BVL-2632915, BVL-2632915, TOX2001-1768	Y	N		Bayer CropScience
KCA 5.2.1 /15	[redacted]	1983	Tests to determine antidote effect against FCR 1272 toxicity in rats [redacted] Bayer CropScience, Report No.: 11854, Edition Number: M-037789-01-1 EPA MRID No.: 00131515 Date: 1983-06-01 GLP/GEP: no, unpublished TOX9401941	Y	N		Bayer CropScience

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KCA 5.2.1 /16	[redacted]	2005	Beta-Cyfluthrin (FCR 4545) - Acute toxicity in the rat after oral administration [redacted] BCS-Irvita, Report No.: AT02686, Edition Number: M-263158-01-1 Date: 2005-12-08 GLP/GEP: yes, unpublished BVL-2632941, BVL-2632941, ASB2014-7720	Y	Y	specifically required by Brazil	BCS-Irvita
KCA 5.2.2 /01	[redacted]	1987	FCR 4545 technical - Study of the acute dermal toxicity to rats (formulation in polyethylene glycol E 400) [redacted] Bayer CropScience, Report No.: 16179, Edition Number: M-065404-01-1 EPA MRID No.: 41244105, 41244106 Date: 1987-11-04 GLP/GEP: yes, unpublished TOX9550259	Y	N		Bayer CropScience
KCA 5.2.2 /02	[redacted]	1987	FCR 4545 technical - Study of the acute dermal toxicity to rats (formulation with Xylene) [redacted] Bayer CropScience, Report No.: 16184, Edition Number: M-065822-01-1 Date: 1987-11-05 GLP/GEP: yes, unpublished TOX9550260	Y	N		Bayer CropScience

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KCA 5.2.2 /04	[redacted]	2005	Beta-Cyfluthrin (FCR 4545) - Acute toxicity in the rat after dermal application [redacted] BCS-Irvita, Report No.: AT02656, Edition Number: M-263229-01-1 Date: 2005-11-28 GLP/GEP: yes, unpublished BVL-2632942, BVL-2632942, ASB2014-7721	Y	Y	specifically required by Brazil	BCS-Irvita
KCA 5.2.3 /01	[redacted]	1985	FCR 4545 (techn.) - Study for acute inhalation toxicity [redacted] Bayer CropScience, Report No.: 13751, Edition Number: M-064938-01-1 Date: 1985-08-20 GLP/GEP: no, unpublished TOX9550261	Y	N		Bayer CropScience
KCA 5.2.3 /02	[redacted]	1988	FCR 4545 (c.n. cyfluthrin K+L, proposed) - Studies for acute inhalation toxicity to the rat to OECD guideline no. 403 [redacted] Bayer CropScience, Report No.: 16911, Edition Number: M-066878-01-1 EPA MRID No.: 41205701 Date: 1988-07-18 GLP/GEP: yes, unpublished TOX9550264	Y	N		Bayer CropScience

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KCA 5.2.3 /03	[redacted]	1996	Determination of Cyfluthrin (FCR 1272) in serum, fat and brain of rats after inhalation exposure or oral administration - Analytical part of study T7058167 [redacted] Bayer CropScience, Report No.: MR-365/95, Edition Number: M-044833-01-1 Date: 1996-01-30 GLP/GEP: yes, unpublished **...also filed: KCA 4.1.2 /78** BVL-2632914, BVL-2632914, TOX2001-1767	Y	N		Bayer CropScience
KCA 5.2.4 /01	[redacted]	1985	FCR 4545 (techn.) - Study for irritant/corrosive effect on skin and eye (rabbit) [redacted] Bayer CropScience, Report No.: 13707, Edition Number: M-064879-01-1 EPA MRID No.: 41205702 Date: 1985-08-09 GLP/GEP: no, unpublished **...also filed: KCA 5.2.5 /01** TOX9550265	Y	N		Bayer CropScience

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KCA 5.2.4 /02	[redacted]	1988	FCR 4545 (c.n. Cyfluthrin K+L. proposed) - Study for sensory irritant potential in the rat (RD50 determination) [redacted] Bayer CropScience, Report No.: 16762, Edition Number: M-066856-01-1 EPA MRID No.: 41205707 Date: 1988-05-31 GLP/GEP: no, unpublished TOX9550263	Y	N		Bayer CropScience
KCA 5.2.4 /03	[redacted]	1988	FCR 4545 (c.n. Cyfluthrin K&L, proposed)- Study for sensory irritant potential in the mouse (RD50 determination) [redacted] Bayer CropScience, Report No.: 16694, Edition Number: M-137092-01-1 EPA MRID No.: 41205706 Date: 1988-05-09 GLP/GEP: no, unpublished TOX9550262	Y	N		Bayer CropScience
KCA 5.2.4 /05	[redacted]	2005	Beta-Cyfluthrin (FCR 4545) - Acute skin irritation/corrosion on rabbits [redacted] BCS-Irvita, Report No.: AT02655, Edition Number: M-263238-01-1 Date: 2005-11-25 GLP/GEP: yes, unpublished BVL-2632944, BVL-2632944, ASB2014-7723	Y	Y	specifically required by Brazil	BCS-Irvita

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KCA 5.2.5 /01	[redacted]	1985	FCR 4545 (techn.) - Study for irritant/corrosive effect on skin and eye (rabbit) [redacted] Bayer CropScience, Report No.: 13707, Edition Number: M-064879-01-1 EPA MRID No.: 41205702 Date: 1985-08-09 GLP/GEP: no, unpublished **...also filed: KCA 5.2.4 /01** TOX9550265	Y	N		Bayer CropScience
KCA 5.2.5 /02	[redacted]	2005	Beta-cyfluthrin (FCR 4545) - Acute eye irritation on rabbits [redacted] BCS-Irvita, Report No.: AT02657, Edition Number: M-263232-01-1 Date: 2005-11-25 GLP/GEP: yes, unpublished BVL-2632945, BVL-2632945, ASB2014-7724	Y	Y	specifically required by Brazil	BCS-Irvita
KCA 5.2.6 /03	[redacted]	2005	Beta-cyfluthrin (FCR 4545) (Project: beta-cyfluthrin technical) - Study for the skin sensitisation effect in guinea pigs (Buehler Patch Test) [redacted] BCS-Irvita, Report No.: AT02683, Edition Number: M-263247-01-1 Date: 2005-11-30 GLP/GEP: yes, unpublished BVL-2632946, BVL-2632946, ASB2014-7725	Y	Y	specifically required by Brazil	BCS-Irvita

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KCA 5.3 /01	El-Elaimy, I.	1986	Biochemical disturbance in liver function and whole blood ache. Due to repeated dermal application of Baythroid to rat Publisher:Faculty of Science, Location:El-Monoufia University, Egypt, Journal:Proc. Zool. Soc. A. R. Egypt., Volume:10, Pages:51-60, Year:1986, Report No.: MO-01-006170, Edition Number: M-048653-01-1 GLP/GEP: n.a., published TOX9401884	Y	N		LIT
KCA 5.3.1 /01	[redacted]	1988	FCR 4545 techn. - Subacute study of oral toxicity to rats [redacted] Bayer CropScience, Report No.: 16384, Edition Number: M-064606-01-1 EPA MRID No.: 41244117 Date: 1988-01-27 GLP/GEP: yes, unpublished TOX9550271	Y	N		Bayer CropScience

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KCA 5.3.1 /02	[redacted]	1996	21-day dermal toxicity study with technical grade Bay-throid in rats [redacted] Bayer CropScience, Report No.: 107437, Edition Number: M-041225-01-1 EPA MRID No.: 44066001 Date: 1996-06-06 GLP/GEP: yes, unpublished TOX2001-1769	Y	N		Bayer CropScience
KCA 5.3.2 /01	[redacted]	1988	FCR 4545 - Subchronic toxicological study on rats (administration with feed for 13 weeks) [redacted] Bayer CropScience, Report No.: 16807, Edition Number: M-137143-02-1 Date: 1988-06-21 **...Amended: 1994-06-21** GLP/GEP: yes, unpublished TOX9550272	Y	N		Bayer CropScience
KCA 5.3.2 /02	[redacted]	1987	FCR 4545 - Study of subchronic oral toxicity to dogs (13-week feeding study) [redacted] Bayer CropScience, Report No.: 16180, Edition Number: M-065806-01-1 Date: 1987-11-04 GLP/GEP: yes, unpublished TOX9550274	Y	N		Bayer CropScience

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KCA 5.3.2 /03	[redacted]	1983	Three-month subacute toxicity study of FCR 1272 in rats [redacted] Bayer CropScience, Report No.: JAP264, Edition Number: M-044018-01-1 Date: 1983-07-31 GLP/GEP: no, unpublished TOX9401881	Y	N		Bayer CropScience
KCA 5.3.2 /04	[redacted]	1984	FCR 1272 (common name: Cyfluthrin, the active ingredient of Baythroid) - Study of the subchronic inhalation toxicity in accordance with OECD guideline no. 413 [redacted] Bayer CropScience, Report No.: 12436, Edition Number: M-037526-03-1 EPA MRID No.: 40082901 Date: 1984-02-01 **...Amended: 1987-07-30** GLP/GEP: yes, unpublished **...also filed: KCA 5.3.3 /04** TOX9401887	Y	N		Bayer CropScience

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KCA 5.3.3 /01	[redacted]	1988	FCR 4545 (common name: Cyfluthrin K+L, suggested) - Study of the range-finding subacute inhalation toxicity to rats in accordance with OECD guideline no. 403 [redacted] Bayer CropScience, Report No.: 16593, Edition Number: M-066752-01-1 EPA MRID No.: 41205708 Date: 1988-04-07 GLP/GEP: no, unpublished TOX9550275	Y	N		Bayer CropScience
KCA 5.3.3 /02	[redacted]	1989	FCR 4545 (c.n.: Betacyfluthrin, proposed) - Subacute inhalation toxicity study in the rat according to OECD guideline no. 412 [redacted] Bayer CropScience, Report No.: 18146, Edition Number: M-137029-01-1 Date: 1989-06-28 GLP/GEP: yes, unpublished TOX9550276	Y	N		Bayer CropScience
KCA 5.3.3 /03	[redacted]	1980	FCR 1272 - Subacute dermal toxicity study on rabbits [redacted] Bayer CropScience, Report No.: 8928, Edition Number: M-039003-01-1 EPA MRID No.: 00131527 Date: 1980-02-05 GLP/GEP: no, unpublished TOX9401883	Y	N		Bayer CropScience

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KCA 5.3.3 /04	[redacted]	1984	FCR 1272 (common name: Cyfluthrin, the active ingredient of Baythroid) - Study of the subchronic inhalation toxicity in accordance with OECD guideline no. 413 [redacted] Bayer CropScience, Report No.: 12436, Edition Number: M-037526-03-1 EPA MRID No.: 40082901 Date: 1984-02-01 **...Amended: 1987-07-30** GLP/GEP: yes, unpublished **...also filed: KCA 5.3.2 /04** TOX9401887	Y	N		Bayer CropScience
KCA 5.3.3 /05	[redacted]	1989	FCR 1272 (common name: cyfluthrin, suggested) - 4-week study of the subacute inhalation toxicity to rats [redacted] Bayer CropScience, Report No.: 18565, Edition Number: M-039780-01-1 EPA MRID No.: 41842601 Date: 1989-11-28 GLP/GEP: yes, unpublished TOX9401886	Y	N		Bayer CropScience

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KCA 5.4 /01	Herbold, B.	1986	FCR 4545 - Salmonella/Microsome test for point-mutagenig effect Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 14187, Edition Number: M-065033-01-1 EPA MRID No.: 41244110 Date: 1986-01-07 GLP/GEP: yes, unpublished TOX9550277	N	N		Bayer CropScience
KCA 5.4 /02	Cifone, M. A.	1987	Mutagenicity test on FCR 4545 technical in the rat primary hepatocyte unscheduled DNA synthesis assay Hazleton Laboratories America, Inc., Kensington, MA, USA Bayer CropScience, Report No.: R4184, Edition Number: M-068738-01-1 EPA MRID No.: 41205704 Date: 1987-09-08 GLP/GEP: yes, unpublished **...also filed: KCA 5.4.1 /03** TOX9550278	N	N		Bayer CropScience

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KCA 5.4 /03	[redacted]	1988	FCR 4545 - Micronucleus test on the mouse to evaluate for clastogenic effects [redacted] Bayer CropScience, Report No.: 16557, Edition Number: M-137089-01-1 EPA MRID No.: 41244111 Date: 1988-03-24 GLP/GEP: yes, unpublished TOX9550279	Y	N		Bayer CropScience
KCA 5.4 /04	[redacted]	2013	Regulatory toxicology - Position paper - Cyfluthrin - BES's position on the genotoxicity potential of cyfluthrin [redacted] Bayer CropScience, Report No.: M-455435-01-1, Edition Number: M-455435-01-1 Date: 2013-05-31 GLP/GEP: n.a., unpublished BVL-2632951, BVL-2632951, ASB2014-7879	Y	Y	Not submitted on EU level for plant protecton products	BCS-Irvita
KCA 5.4.1 /02	Lehn, H.	1988	FCR 4545 (C.N. cyfluthrine K+L (proposed)) - Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT assay *in vitro* Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 16835, Edition Number: M-066869-01-1 EPA MRID No.: 41244112 Date: 1988-06-27 GLP/GEP: yes, unpublished TOX9550280	N	N		Bayer CropScience

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KCA 5.4.1 /03	Cifone, M. A.	1987	Mutagenicity test on FCR 4545 technical in the rat primary hepatocyte unscheduled DNA synthesis assay Hazleton Laboratories America, Inc., Kensington, MA, USA Bayer CropScience, Report No.: R4184, Edition Number: M-068738-01-1 EPA MRID No.: 41205704 Date: 1987-09-08 GLP/GEP: yes, unpublished **...also filed: KCA 5.4 /02** TOX9550278	N	N		Bayer CropScience
KCA 5.4.1 /04	Herbold, B.	2008	Beta-cyfluthrin [project: FCR 4545 (AE 1430672)] - Salmonella/microsome test - Plate incorporation and preincubation method Bayer HealthCare AG, Wuppertal, Germany BCS-Irvita, Report No.: AT04856, Edition Number: M-308394-01-1 Date: 2008-09-22 GLP/GEP: yes, unpublished BVL-2632948, BVL-2632948, ASB2014-7875	N	Y	new data requirement	BCS-Irvita

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KCA 5.5 /02	[redacted]	1997	Technical grade Cyfluthrin (FCR 1272) - A chronic toxicity feeding study in the beagle dog [redacted] Bayer CropScience, Report No.: BC8365, Edition Number: M-044511-02-1 EPA MRID No.: 45189201 Date: 1997-11-10 **...Amended: 2000-07-20** GLP/GEP: yes, unpublished TOX9800225	Y	N		Bayer CropScience
KCA 5.5 /04	[redacted]	1997	Technical grade Cyfluthrin - A combined chronic toxicity/oncogenicity testing study in the rat [redacted] Bayer CropScience, Report No.: BC8384, Edition Number: M-044524-02-1 EPA MRID No.: 44459301 Date: 1997-12-12 **...Amended: 2000-07-19** GLP/GEP: yes, unpublished TOX9850068	Y	N		Bayer CropScience

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KCA 5.6.1 /02	[redacted]	1996	A two-generation reproduction study in rats using technical grade Cyfluthrin administered via the diet [redacted] Bayer CropScience, Report No.: BC7910, Edition Number: M-032017-01-1 EPA MRID No.: 44371401 Date: 1996-03-08 GLP/GEP: yes, unpublished TOX2001-1771	Y	N		Bayer CropScience
KCA 5.6.1 /03	[redacted]	1997	A supplementary two-generation dietary reproduction study in rats using technical grade Cyfluthrin [redacted] Bayer CropScience, Report No.: BC8077, Edition Number: M-032020-01-1 EPA MRID No.: 44371402 Date: 1997-01-30 GLP/GEP: yes, unpublished TOX2001-1772	Y	N		Bayer CropScience
KCA 5.6.2 /01	[redacted]	1982	FCR 1272 - Evaluation for embryotoxic and teratogenic effects on orally dosed rats [redacted] Bayer CropScience, Report No.: 10562, Edition Number: M-037361-01-1 EPA MRID No.: 00131533 Date: 1982-01-20 GLP/GEP: no, unpublished TOX9401908	Y	N		Bayer CropScience

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KCA 5.6.2 /02	[redacted]	1983	Embryotoxicity (including teratogenicity) study with FCR 1272 in the rat [redacted] Bayer CropScience, Report No.: R2774, Edition Number: M-039488-01-1 EPA MRID No.: 00157794, 00157883 Date: 1983-12-14 GLP/GEP: yes, unpublished TOX9401909	Y	N		Bayer CropScience
KCA 5.6.2 /03	[redacted]	1996	A developmental toxicity study with FCR 4545 technical in the Wistar rat [redacted] Bayer CropScience, Report No.: BC7989, Edition Number: M-136592-01-1 EPA MRID No.: 44116501 Date: 1996-09-04 GLP/GEP: yes, unpublished TOX2001-1773	Y	N		Bayer CropScience

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KCA 5.6.2 /04	[redacted]	1988	FCR 1272 (Cyfluthrin) - Study for embryotoxic effects on rats after inhalation [redacted] Bayer CropScience, Report No.: 16391, Edition Number: M-041542-02-1 EPA MRID No.: 40968501, 42332001 Date: 1988-02-01 **...Amended: 1988-08-16** GLP/GEP: yes, unpublished TOX9401910	Y	N		Bayer CropScience
KCA 5.6.2 /05	[redacted]	1993	FCR 1272 - Determination of FCR 1272 concentration in the plasma of rats following inhalative exposure [redacted] Bayer CropScience, Report No.: 22726, Edition Number: M-038776-01-1 Date: 1993-12-02 GLP/GEP: no, unpublished TOX9401913	Y	N		Bayer CropScience
KCA 5.6.2 /06	[redacted]	1993	FCR 1272 (c.n. Cyfluthrin) - Inhalation study for embryotoxic effects in rats [redacted] Bayer CropScience, Report No.: 22581, Edition Number: M-038947-01-1 Date: 1993-10-05 GLP/GEP: yes, unpublished TOX9401829	Y	N		Bayer CropScience

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KCA 5.6.2 /07	[redacted]	1983	FCR 1272 (proposed common name cyfluthrin) - Study for embryotoxic effects on rabbits after oral administration [redacted] Bayer CropScience, Report No.: 11855, Edition Number: M-037892-01-1 EPA MRID No.: 00131534 Date: 1983-06-01 GLP/GEP: no, unpublished TOX9401914	Y	N		Bayer CropScience
KCA 5.6.2 /08	[redacted]	1992	Embryotoxicity study (including teratogenicity) with FCR 1272 in the rabbit [redacted] Bayer CropScience, Report No.: R5770, Edition Number: M-039695-01-1 EPA MRID No.: 42675401 Date: 1992-12-03 GLP/GEP: yes, unpublished TOX9401915	Y	N		Bayer CropScience
KCA 5.6.2 /09	[redacted]	1992	FCR 1272 (c.n.: Cyfluthrin) - Pilot study for acid-base status following inhalation exposure to the rat [redacted] Bayer CropScience, Report No.: 21865, Edition Number: M-038738-01-1 Date: 1992-11-24 GLP/GEP: yes, unpublished TOX9401940	Y	N		Bayer CropScience

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KCA 5.7.1 /01	[redacted]	1982	FCR 1272 - Study for nerve damage effect on the rat after 5-months oral application [redacted] Bayer CropScience, Report No.: 10705, Edition Number: M-037737-01-1 EPA MRID No.: 00131529 Date: 1982-03-10 GLP/GEP: no, unpublished TOX9401922	Y	N		Bayer CropScience
KCA 5.7.1 /02	[redacted]	1983	FCR 1272 (proposed common name cyfluthrin) - Study for neurotoxic effect on rats after subacute oral administration [redacted] Bayer CropScience, Report No.: 12338, Edition Number: M-037482-02-1 EPA MRID No.: 00156957 Date: 1983-12-27 **...Amended: 1985-10-08** GLP/GEP: no, unpublished TOX9401924	Y	N		Bayer CropScience

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KCA 5.7.1 /03	[redacted]	1983	FCR 1272 - Special toxicology study (morphological effects on the nervous system of rats) [redacted] Bayer CropScience, Report No.: R3362, Edition Number: M-074939-01-1 Date: 1983-06-30 GLP/GEP: no, unpublished TOX9401923	Y	N		Bayer CropScience
KCA 5.7.1 /04	[redacted]	2003	A developmental neurotoxicity screening study with technical grade beta-cyfluthrin in Wistar rats [redacted] BCS-Irvita, Report No.: 200620, Edition Number: M-103213-01-1 EPA MRID No.: 46054101 Date: 2003-07-29 GLP/GEP: yes, unpublished BVL-2632952, BVL-2632952, ASB2007-2856	Y	Y	specifically required by US	BCS-Irvita
KCA 5.7.2 /01	[redacted]	1981	FCR 1272 - NAK 1472 - NAK 1654 - Investigative neurotoxicity studies in hens [redacted] Bayer CropScience, Report No.: BC165, Edition Number: M-038984-01-1 EPA MRID No.: 00131544 Date: 1981-03-09 GLP/GEP: no, unpublished TOX9401918	Y	N		Bayer CropScience

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KCA 5.7.2 /02	[redacted]	1981	FCR 1272 - Neurotoxicity studies on hens [redacted] Bayer CropScience, Report No.: 9753, Edition Number: M-030275-01-2 EPA MRID No.: 00131543 Date: 1981-01-27 GLP/GEP: no, unpublished TOX9401916	Y	N		Bayer CropScience
KCA 5.7.2 /03	[redacted]	1985	Commentary on report no. 9753 of 27.01.1981 (FCR 1272 - Neuotoxic study with chickens by Dr. J. Thyssen, Dr. G. Kaliner and Dr. P. Groening) [redacted] Bayer CropScience, Report No.: MO-01-011575, Edition Number: M-051937-01-1 EPA MRID No.: 00157800 Date: 1985-04-09 GLP/GEP: no, unpublished TOX9401917	Y	N		Bayer CropScience
KCA 5.7.2 /04	[redacted]	1986	Acute delayed neurotoxicity study with FCR 1272 (c.n. Cyfluthrin) in the hen [redacted] Bayer CropScience, Report No.: R3690, Edition Number: M-039448-01-1 EPA MRID No.: 00163040 Date: 1986-04-11 GLP/GEP: yes, unpublished TOX9401920	Y	N		Bayer CropScience

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KCA 5.7.2 /05	[redacted]	1985	FCR 1272 (c.n. cyfluthrin) - Study for effect on the neurotoxic target enzyme (NTE) with the chicken (gallus domesticus) [redacted] Bayer CropScience, Report No.: 13821, Edition Number: M-040975-01-1 EPA MRID No.: 00156585 Date: 1985-09-16 GLP/GEP: no, unpublished TOX9401919	Y	N		Bayer CropScience
KCA 5.7.2 /06	[redacted]	1982	FCR 1272 (Cyfluthrin, Baythroid active ingredient) - Neurotoxicity study on chickens after cutaneous administration (cumulation tests) [redacted] Bayer CropScience, Report No.: 10768, Edition Number: M-037549-01-1 EPA MRID No.: 00131545 Date: 1982-03-29 GLP/GEP: no, unpublished TOX9401921	Y	N		Bayer CropScience

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KCA 5.7.2 /07	[redacted]	1983	FCR 1272 (Baythroid active ingredient) (Common name: cyfluthrin) - Study for acute and subacute inhalation toxicity on chickens [redacted] Bayer CropScience, Report No.: 11558, Edition Number: M-037613-01-1 EPA MRID No.: 00131510 Date: 1983-02-14 GLP/GEP: no, unpublished TOX9401865	Y	N		Bayer CropScience
KCA 5.7.2 /08	[redacted]	1997	A subchronic dietary neurotoxicity screening study with technical grade FCR 4545 (Beta-Cyfluthrin) in Fischer 344 rats [redacted] Bayer CropScience, Report No.: BC8157, Edition Number: M-038537-01-1 EPA MRID No.: 44296001 Date: 1997-05-09 GLP/GEP: yes, unpublished **...also filed: KCA 5.8 /14** TOX2001-1266	Y	N		Bayer CropScience

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KCA 5.8 /01	[redacted]	1985	FCR 4545 - Range-Finding test for acute toxicity to the dog [redacted] Bayer CropScience, Report No.: 13726, Edition Number: M-064907-01-1 Date: 1985-08-14 GLP/GEP: no, unpublished **...also filed: KCA 5.2.1 /07** TOX9550254	Y	N		Bayer CropScience
KCA 5.8 /02	[redacted]	1987	FCR 4545 technical - Study of the acute intraperitoneal toxicity to rats (formulation in polyethylene glycol E 400) [redacted] Bayer CropScience, Report No.: 16104, Edition Number: M-065308-01-1 EPA MRID No.: 41244113 Date: 1987-10-13 GLP/GEP: yes, unpublished **...also filed: KCA 5.8.2 /02** TOX9550269	Y	N		Bayer CropScience

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KCA 5.8 /03	[redacted]	1988	FCR 4545, FCR 1272 - Comparative study of the acute intraperitoneal toxicity to mice (formulation in polyethylene glycol E 400) [redacted] Bayer CropScience, Report No.: 17423, Edition Number: M-038567-01-1 EPA MRID No.: 41244114 Date: 1988-11-25 GLP/GEP: yes, unpublished TOX9550270	Y	N		Bayer CropScience
KCA 5.8 /04	[redacted]	1981	3-phenoxy-4-fluoro-benzaldehyde (intermediate for FCR 1272) - Industrial toxicity studies [redacted] Bayer CropScience, Report No.: 9942, Edition Number: M-037220-01-1 Date: 1981-05-08 GLP/GEP: no, unpublished TOX9401927	Y	N		Bayer CropScience
KCA 5.8 /05	Herbold, B.	1985	4-fluoro-3-phenoxybenzaldehyde (= FPBA) - Salmonella/microsome test to evaluate for point mutation Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 13429, Edition Number: M-040955-01-1 EPA MRID No.: 00157797 Date: 1985-04-22 GLP/GEP: no, unpublished TOX9401928	N	N		Bayer CropScience

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KCA 5.8 /06	Herbold, B.	1987	RAD 69/86 - Salmonella/microsome test for point-mutagenetic effect Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 15724, Edition Number: M-037991-01-1 Date: 1987-04-21 GLP/GEP: no, unpublished TOX9401935	N	N		Bayer CropScience
KCA 5.8 /07	Herbold, B. A.	1988	FCR 2947 - Salmonella/microsome test to evaluate for point mutagenetic effects Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 16703, Edition Number: M-038335-01-1 Date: 1988-05-11 GLP/GEP: yes, unpublished TOX9401938	N	N		Bayer CropScience
KCA 5.8 /11	[redacted]	1982	Safety pharmacology study with FCR 1272 on oral administration [redacted] Bayer CropScience, Report No.: R2405, Edition Number: M-039504-01-1 Date: 1982-12-01 GLP/GEP: yes, unpublished TOX9401943	Y	N		Bayer CropScience

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KCA 5.8 /12	[redacted]	1999	Cyfluthrin (c.n.: Cyfluthrin) - Special study for acute oral toxicity in rats (slip angle test) [redacted] Bayer CropScience, Report No.: 29371, Edition Number: M-035139-01-1 Date: 1999-12-13 GLP/GEP: yes, unpublished TOX2001-1264	Y	N		Bayer CropScience
KCA 5.8 /13	[redacted]	1997	An acute oral neurotoxicity screening study with technical grade FCR 4545 in Fischer 344 rats [redacted] Bayer CropScience, Report No.: BC8265, Edition Number: M-038521-01-1 EPA MRID No.: 44401101 Date: 1997-10-02 GLP/GEP: yes, unpublished TOX2001-1265	Y	N		Bayer CropScience
KCA 5.8 /14	[redacted]	1997	A subchronic dietary neurotoxicity screening study with technical grade FCR 4545 (Beta-Cyfluthrin) in Fischer 344 rats [redacted] Bayer CropScience, Report No.: BC8157, Edition Number: M-038537-01-1 EPA MRID No.: 44296001 Date: 1997-05-09 GLP/GEP: yes, unpublished **...also filed: KCA 5.7.2 /08** TOX2001-1266	Y	N		Bayer CropScience

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KCA 5.8 /15	[redacted]	1982	FCR 1272 and SIR 8514 - Study for acute combination toxicity [redacted] Bayer CropScience, Report No.: 10516, Edition Number: M-037360-01-1 Date: 1982-01-15 GLP/GEP: no, unpublished TOX9401946	Y	N		Bayer CropScience
KCA 5.8 /16	[redacted]	1983	FCR 1272 & SRA 5172 (c. n. cyfluthrin (proposed) and methamidophos) - Study for combination toxicity [redacted] Bayer CropScience, Report No.: 12003, Edition Number: M-037421-01-1 Date: 1983-08-17 GLP/GEP: no, unpublished TOX9401947	Y	N		Bayer CropScience
KCA 5.8 /17	[redacted]	1984	FCR 1272 (cyfluthrin) BOQ 5812315 (propoxur) - Study for combination toxicity [redacted] Bayer CropScience, Report No.: 12544, Edition Number: M-040810-01-1 Date: 1984-03-14 GLP/GEP: no, unpublished TOX9401948	Y	N		Bayer CropScience

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KCA 5.8 /18	[redacted]	1984	FCR 1272 (cyfluthrin) DDVP (dichlorvos) - Study for combination toxicity [redacted] Bayer CropScience, Report No.: 12567, Edition Number: M-040813-01-1 Date: 1984-03-27 GLP/GEP: no, unpublished TOX9401949	Y	N		Bayer CropScience
KCA 5.8 /19	[redacted]	1994	NTN 33893 (c.n. Imidacloprid [proposed]), FCR 1272 (c.n. cyfluthrin) - Study for combination toxicity in rats [redacted] Bayer CropScience, Report No.: 23420, Edition Number: M-029146-02-1 Date: 1994-10-19 **...Amended: 1995-02-02** GLP/GEP: yes, unpublished TOX2001-1764	Y	N		Bayer CropScience
KCA 5.8 /20	[redacted]	1988	E 6876 and FCR 1272 (c.n. omethoate, cyfluthrin) - Study for combination toxicity to rats [redacted] Bayer CropScience, Report No.: 16968, Edition Number: M-038366-01-1 Date: 1988-07-28 GLP/GEP: no, unpublished TOX9401950	Y	N		Bayer CropScience

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KCA 5.8 /21	Waerngard, L.; Flodstroem, S.	1989	Effects of Tetradecanoyl phorbol acetate, pyrethroids and DDT in the V79 Publisher:Anon., Location:Anon., Journal:Cell Biology and Toxicology, Volume:5, Issue:1, Pages:67-75, Year:1989, Report No.: MO-01-006661, Edition Number: M-049078-01-1 GLP/GEP: n.a., published TOX9401951	N	N		LIT
KCA 5.8 /22	Crofton, K. M.; Reiter, L. W.	1988	The effects of type I and II pyrethroids on motor activity and the acoustic startle response in the rat Publisher:Society of Toxicology, Location:Anon., Journal:Fundamental and Applied Toxicology, Volume:10, Pages:624-634, Year:1988, Report No.: MO-01-006166, Edition Number: M-048649-01-1 GLP/GEP: n.a., published TOX9401952	Y	N		LIT

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KCA 5.8 /23	[redacted]	1998	Safety and tolerability study of FCR 1272 0.04 AE in healthy volunteers [redacted] Bayer CropScience, Report No.: 11590, Edition Number: M-031568-01-1 Date: 1998-10-07 GLP/GEP: yes, unpublished TOX2001-879	Y	N		Bayer CropScience
KCA 5.8 /24	Pauluhn, J.	1996	Risk assessment of pyrethroids following indoor use Publisher:Elsevier Ireland Ltd., Location:Ireland, Journal:Toxicology Letters, Volume:88, Pages:339-348, Year:1996, Report No.: Lit. 1560, Edition Number: M-034669-01-1 GLP/GEP: n.a., published TOX2001-880	Y	N		LIT
KCA 5.8 /25	[redacted]	1998	Technical grade Cyfluthrin - An oncogencity testing study in the mouse [redacted] Bayer CropScience, Report No.: BC8492, Edition Number: M-027231-02-1 EPA MRID No.: 45228101 Date: 1998-05-28 **...Amended: 2000-09-06** GLP/GEP: yes, unpublished TOX2001-1770	Y	N		Bayer CropScience

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KCA 5.8.1 /01	[redacted]	1982	Comparative study on inhibition of the Na+, K+ and Mg++-dependent ATPase from rats and chickens brains *in vitro* by FCR 1272, some of its metabolites and further substances such as DDT, Ouabain, some pyrethroids and phosphoric acid esters [redacted] Bayer CropScience, Report No.: 11116, Edition Number: M-037564-01-1 Date: 1982-08-27 GLP/GEP: no, unpublished TOX9401939	Y	N		Bayer CropScience
KCA 5.8.1 /02	Herbold, B.	1987	FCR 1261 - Salmonella/microsome test for point-mutagenic effect Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: 15909, Edition Number: M-038181-01-1 Date: 1987-07-03 GLP/GEP: no, unpublished TOX9401936	N	N		Bayer CropScience

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KCA 5.8.2 /02	[redacted]	1987	FCR 4545 technical - Study of the acute intraperitoneal toxicity to rats (formulation in polyethylene glycol E 400) [redacted] Bayer CropScience, Report No.: 16104, Edition Number: M-065308-01-1 EPA MRID No.: 41244113 Date: 1987-10-13 GLP/GEP: yes, unpublished **...also filed: KCA 5.8 /02** TOX9550269	Y	N		Bayer CropScience
KCA 5.8.2 /03	[redacted]	1982	FCR 1272 - Comparative tests for acute toxicity with various formulation aids [redacted] Bayer CropScience, Report No.: 10931, Edition Number: M-021687-01-1 EPA MRID No.: 00131518 Date: 1982-06-07 GLP/GEP: no, unpublished TOX9401854	Y	N		Bayer CropScience
KCA 5.9 /01	Faul, J.; Krauthausen, E.	1995	FCR 1272 - Occupational medical experience Bayer AG, Dormagen, Germany Bayer CropScience, Report No.: MO-01-005435, Edition Number: M-046951-01-1 Date: 1995-04-01 GLP/GEP: no, unpublished TOX2005-1679	N	N		Bayer CropScience

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KCA 5.9 /02	Steffens, W.	2014	Occupational medical experiences with beta-cyfluthrin BCS-Irvita, Report No.: M-476492-01-1, Edition Number: M-476492-01-1 Date: 2014-01-20 GLP/GEP: no, unpublished **...also filed: KCA 3.10 /02** BVL-2632953, BVL-2632953, ASB2014-7889	N	Y	data not submitted on EU level	BCS-Irvita
KCA 5.9.1 /01	Flucke, W.	1979	Memorandum - Irritant effects after work with FCR 1272 Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: MO-01-005453, Edition Number: M-046997-01-1 Date: 1979-08-10 GLP/GEP: no, unpublished TOX9401953	Y	N		Bayer CropScience
KCA 5.9.1 /02	Miksche, L.	1979	Symptoms of irritation when working with FCR 1272 Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: MO-01-005449, Edition Number: M-046994-01-1 Date: 1979-08-21 GLP/GEP: no, unpublished TOX9401954	Y	N		Bayer CropScience

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KCA 5.9.1 /03	Faul, J.	1984	Flubenzimine (SLJ 0312); Triazoxide (SAS 9244); Cyfluthrin (FCR 1272) Bayer AG, Dormagen, Germany Bayer CropScience, Report No.: MO-01-005447, Edition Number: M-046978-01-1 Date: 1984-07-02 GLP/GEP: no, unpublished TOX9401957	Y	N		Bayer CropScience
KCA 5.9.1 /04	Faul, J.	1988	Medical data on employees in Cyfluthrin formulation Bayer AG, Dormagen, Germany Bayer CropScience, Report No.: MO-01-005441, Edition Number: M-046968-01-1 Date: 1988-03-28 GLP/GEP: no, unpublished TOX9401959	Y	N		Bayer CropScience
KCA 5.9.1 /05	Faul, J.	1995	FCR 1272 - Occupational medical experience Bayer AG, Dormagen, Germany Report No.: MO-04-009035, Edition Number: M-088092-01-1 Date: 1995-04-01 GLP/GEP: no, unpublished TOX2005-1679	Y	N		Bayer CropScience

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KCA 5.9.1 /06	Kollert, W.	1988	Medical data on workers employed in cyfluthrin production and formulation/registration of the active ingredient in Brazil Bayer AG, Wuppertal, Germany Bayer CropScience, Report No.: MO-01-005442, Edition Number: M-046972-01-1 Date: 1988-03-02 GLP/GEP: no, unpublished TOX9401958	Y	N		Bayer CropScience
KCA 5.9.1 /07	Krauthausen, E.	1995	FCR 1272 - Occupational medical experience Bayer AG, Dormagen, Germany Report No.: MO-04-009036, Edition Number: M-088095-01-1 Date: 1995-04-01 GLP/GEP: no, unpublished ASB2009-987	Y	N		Bayer CropScience
KCA 5.9.1 /08	Vijverberg, H. P. M.; van den Bercken, J.	1982	Action of pyrethroid insecticides on the vertebrate nervous system Publisher:Blackwell Scientific Publications, Location:Anon., Journal:Neuropathology and Applied Neurobiology, Volume:8, Issue:Anon., Pages:421-440, Year:1982, Report No.: MO-01-006660, Edition Number: M-049076-01-1 GLP/GEP: n.a., published TOX9401956	Y	N		LIT

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KCA 5.9.1 /09	Narahashi, T.	1989	Cellular mechanisms of Pyrethroids Publisher:Gordon and Breach Science Publishers S. A., Location:Great Britain, Journal:Comments Toxicology, Volume:3, Issue:5, Pages:363-379, Year:1989, Report No.: MO-01-006463, Edition Number: M-048889-01-1 GLP/GEP: n.a., published TOX9401960	Y	N		LIT
KCA 5.9.1 /10	Aldridge, W. N.	1990	An assessment of the toxicological properties of pyrethroids and their neurotoxicity Publisher:Anon., Location:Anon., Journal:Critical reviews in toxicology, Volume:21, Issue:2, Pages:89-104, Year:1990, Report No.: MO-01-006152, Edition Number: M-048635-01-1 GLP/GEP: n.a., published TOX9401961	Y	N		LIT

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KCA 5.9.1 /11	Vijverberg, H. P. M.; van den Bercken, J.	1990	Neurotoxicological effects and the mode of action of pyrethroid in insecticides Publisher:CRC Press, Inc., Location:Anon., Journal:Critical Reviews in Toxicology, Volume:21, Issue:2, Pages:105-126, Year:1990, Report No.: MO-01-006651, Edition Number: M-049068-01-1 GLP/GEP: n.a., published TOX9401962	Y	N		LIT
KCA 5.9.1 /12	Quesne, P. M.; Maxwell, I. C.; Butterworth, S. T. G.	1980	Transient facial sensory symptoms following exposure to synthetic pyrethroids: a clinical and electrophysiological assessment Publisher:Pathotox Publishers, Inc., Location:Great Britain, Journal:Neurotoxicology, Volume:2, Pages:1-11, Year:1980, Report No.: MO-01-006497, Edition Number: M-048914-01-1 GLP/GEP: n.a., published TOX9401955	Y	N		LIT

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KCA 5.9.3 /01	He, F.; Wang, S.; Liu, L.; Chen, S.; Zhang, Z.; Sun, J.	1989	Clinical manifestations and diagnosis of acute pyrethroid poisoning Publisher:Springer-Verlag, Journal:Archives of Toxicology, Volume:63, Pages:54-58, Year:1989, Report No.: MO-01-006440, Edition Number: M-048869-01-1 GLP/GEP: n.a., published TOX9401963	Y	N		LIT
KCA 5.1.2	Anadón, A.; Martínez, M. A.; Martínez, M; Castellano, V.; Ares, I.; Romero, A.; Fernández, R.; Martínez-Larranaga, M. R.	2013	Differential induction of cytochrome P450 isoforms and peroxisomal proliferation by Cyfluthrin in male wistar rats Page: 135– 142 Toxicology Letters (2013) 135– 142 ASB2015-926 BVL-3094365	J	N		LIT
KCA 5.2.1	Bhushan B.; Saxena P. N.; Saxena. N.	2013	Biochemical and histological changes in rat liver caused by Cypermethrin and beta-Cyfluthrin Page:57-67 ! DOI: 10.2478/10004-1254-64-2013-2184 Arh Hig Rada Toksicol 2013, 64 (2013) 57-67 ASB2015-644 BVL-3094357	J	N		LIT
KCA 5.3.3	Bhushan B.; Saxena P. N.; Saxena. N.		Biochemical and histological changes in rat liver caused by Cypermethrin and beta-Cyfluthrin Page:57-67 ! DOI: 10.2478/10004-1254-64-2013-2184 Arh Hig Rada Toksicol 2013, 64 (2013) 57-67 ASB2015-644 BVL-3137975	J	N		LIT

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KCA 5.3.3	Bhushan, B; Saxena, N.; Saxena, P. N.	2010	Beta-Cyfluthrin induced histochemical alterations in the liver of the albino rat page 61-66 Scand. J. Lab. vol.Vol.32, No.2 (2010) 61-66 ASB2015-1098 BVL-3094358	J	N		LIT
KCA 5.7.1	Breckenridge, C. B.; Holden, L.; Sturgess, N.; Weiner, M.; Sheets, L.; Sargent, D.; Soderlund, D. M.; Sung Choi, J-S.; Symington, S.; Clark, J. M.; Burr, S.; Ray. D.	2009	Evidence for a separate mechanism of toxicity for the Type I and the Type II Pyrethroid insecticides Page: S17–S31 NeuroToxicology (2009) S17–S31 ASB2015-930 BVL-3094359	J	N		LIT
KCA 5.7.1	Clark, M. J.; Symington, S. B.	2008	Neurotoxic implications of the agonistic action of CS-syndrome pyrethroids on the N-type Cav2.2 calcium channel Page: 628–638 ! DOI: 10.1002/ps Pest Management Science (2008) 628–638 ASB2015-919 BVL-3094366	J	N		LIT

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KCA 5.7.1	Guvenc, D.; Aksoy, A.; Gacar, A.; Atmaca, E.; Kursad, A.; Dasa, Y.; Guvenc, T.	2014	Evaluation of changes in monoamine levels and apoptosis induced by Cyfluthrin in rats Page: 331–340 ! DOI: 10.1039/c4tx00041b Toxicology Research (2014) ASB2015-923 BVL-3094367	J	N		LIT
KCA 5.3.3	Jebur, A. B.; Nasr, H. M.; Demerdash, F. El.	2013	Selenium modulates b-Cyfluthrin - induced liver oxidative toxicity in rats Page: 1223-1329 ! DOI 10.1002/tox Environmental Toxicology ASB2015-921 BVL-3094368	J	N		LIT
KCA 5.9	~~Kern County~~ Anonymous	2006	Worker illness related to ground application of pesticide Page: 486-488 ASB2015-929 BVL-3094360	N	N		LIT
KCA 5.9	Miller, M. A.; Menowsky, M.	2014	Human intravenous injection of b-cyfluthrin with minimal toxic effects Page: 113.e1–113.e2 American Journal of Emergency Medicine (2014) 113.e1–113.e2 ASB2015-928 BVL-3094361	J	N		LIT

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KCA 5.8	Sadowska-Woda, I.; Wójcik, N.; Karowicz-bilinska, A,; Bieszczad-bedrejczuk, E.	2009	Effect of selected antioxidants in b-Cyfluthrin-induced oxidative stress in human erythrocytes *in vitro* Page: 879–884 Toxicology *in vitro* (2010) ASB2015-790 BVL-3094369	N	N		LIT
KCA 5.1.2	Scollon, E. J.; Starr, J. M.; Godin, S. J.; DeVito, M. J.; Hughes, M. F.	2008	*In vitro* metabolism of Pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 Isoforms Page: 221–228 ! 22343/3422733 DRUG METABOLISM AND DISPOSITION vol.37, 1 (2008) 221–228 ASB2015-931 BVL-3094362	N	N		LIT
KCA 5.7.1	Weiner, M. L.; Nemec, M.; Sheets, L.; Sargent, D.; Breckenridge, C.	2009	Comparative functional observational battery study of twelve commercial Pyrethroid insecticides in male rats following acute oral exposure Page: S1–S16 NeuroToxicology (2009) ASB2015-934 BVL-3094363	J	N		LIT
KCA 5.8	Yilmaz, M; Rencuzo-gullarim, E.; Canli, M.	2014	The effects of Cyfluthrin on some biomarkers in the liver and kidney of wistar rats DOI 10.1007/s11356-014-3734-6 Springer-Verlag (2014) ASB2015-888 BVL-3094370	J	N		LIT

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KCA 5.5	Zhang, J.; Zhu, W.; Zheng, Y.; Yang, J.; Zhu, X.	2008	The antiandrogenic activity of pyrethroid pesticides Cyfluthrin and b-Cyfluthrin Page: 491–496 Reproductive Toxicology (2008) ASB2015-918 BVL-3094364	J	N		LIT
EG: P-MCA p	Anon.	2014	beta-Cyfluthrin: P-MCA Section 9 - Literature data M-483598-01-1 ! P-MCA / Sec. 9 ASB2014-7922 BVL-2633106	N	N		Beta-Cyfluthrin Task Force

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