European Commission

TRANSFLUTHRIN

CAS number 118712-89-3

Document III-A
Section 6 Toxicology
Study Summaries
Active Substance

Rapporteur Member State: The Netherlands
August 2013

CA-report and Proposed Decision of The Netherlands in the context of the
Possible inclusion of Transfluthrin in Annex I of Council Directive 98/8/EC

CONTENTS

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Acute Toxicity

Acute oral toxicity in the mouse

SECTION A6.1.1

BPD Data set IIA/
Annex Point VI.6.1.1

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		1 REFERENCE
1.1	**Reference**	[redacted] (1988a).
		NAK 4455 techn. Study for acute oral toxicity to mice [redacted].
		[redacted] Report No. T 4025025 [BES Ref: MO-03-009933]
		Report date: September 14, 1988
		Unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience
1.2.2	Companies with letters of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes
		OECD 401 (1981)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	NAK 4455 (transfluthrin)
3.1.1	Lot/Batch number	130187
3.1.2	Specification	As given in sections 2 and 3
3.1.2.1	Description	Dark brown solid/liquid
3.1.2.2	Purity	94.5%
3.1.2.3	Stability	Test substance was stored in a laboratory cabinet (at 21.5-24°C) and kept stable throughout the study.

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3.2	**Test Animals**	
3.2.1	Species	Mouse
3.2.2	Strain	NMRI (SPF-Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and female
3.2.5	Age/weight at study initiation	4 – 5 weeks Weight range of 20 – 28 g (males) and 20 – 25 g (females)
3.2.6	Number of animals per group	5 mice/sex/group
3.2.7	Control animals	No
3.3	**Administration/ Exposure**	Oral
3.3.1	Postexposure period	14 days
		Oral
3.3.2	Type	Gavage to fasted animals (16 hours)
3.3.3	Concentration	Males: 100, 160, 250, 500, 630, 710, 1000, 1600 and 5000 mg/kg bw Females: 100, 250, 500, 630, 710, 1000 and 5000 mg/kg bw
3.3.4	Vehicle	Polyethylene glycol E 400
3.3.5	Concentration in vehicle	Not stated
3.3.6	Total volume applied	5 or 10 mL/kg
3.3.7	Controls	None
3.4	**Examinations**	Clinical observations, necropsy, body weight
3.5	**Method of determination of LD_{50}**	Rosiello, Essigmann and Wogan as modified by Pauluhn based on Bliss, Litchfield and Wilcoxon, Finney, Weil, Thompson, Miller and Tainter
3.6	**Further remarks**	None

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		4 RESULTS AND DISCUSSION	
4.1	**Clinical signs**	At 250 mg/kg and higher most of the animals died within 24 hours after dosing. No mortality was observed in animals at 100 and 160 mg/kg. Clinical signs included apathy in all groups except 100 mg/kg bw for both males and females. At 250 mg/kg bw tremor was observed in both sexes, additionally females exhibited prostration on the side. At higher doses, spasmodic tremor, dyspnoea and bristling coasts also appeared. The symptoms were apparent for a maximum of five days after administration. No body weight changes were observed during the observation period.	X
4.2	**Pathology**	No abnormal findings in animals sacrificed at end of study. In animals that died during the study, the following observations were made: lung patchy, distended; liver pale, patchy lobulation; isolated spleens and kidneys pale; in isolated cases dark mucus in the stomach or stomach distended.	
4.3	**Other**	No other significant effects	
4.4	**LD_{50}**	LD_{50} (male): 583 mg/kg bw LD_{50} (female): 688 mg/kg bw	

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5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test material is NAK 4455, Batch no. 130187, and has a purity of 94.5%. The study was carried out against the following guidelines: OECD Guideline for Testing of Chemicals, Section 4: Health Effects, No. 401 – "Acute Oral Toxicity", adopted 12th May 1981.

A single dose of the test material made up in polyethylene glycol E 400 was administered by gavage to groups of fasted male and female NMRI (SPF-Han) mice at doses of 100, 160 (male only), 250, 500, 630, 710, 1000, 1600 (male only) and 5000 mg/kg bw.

Clinical signs and bodyweight were recorded for 14 days post administration. All animals were subjected to macroscopic examination at death.

5.2 Results and discussion

At 250 mg/kg and higher most of the animals died within 24 hours after dosing. At 160 mg/kg and higher, the symptoms observed point to an effect of the test compound on the nervous system. These symptoms were apparent for a maximum of five days after administration and disappeared rapidly during the observation period. A dose of 100 mg/kg bw appeared to be well tolerated with no symptoms.

No effects on body weight gain were observed.

Animals that died during the study had some organs that were pale, patchy and/or distended.

The LD50 was calculated to be 583 mg/kg bw for males and 688 mg/kg bw for females.

Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that a classification of 'harmful' is warranted.

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

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	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24-01-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	4.1 first sentence is confusing. Sentence should read something like: Of the animals that died at doses of 250 mg/kg bw or higher, most animals died within 24h after dosing. Otherwise the version of the applicant is adopted
Conclusion	The version of the applicant is adopted; LD_{50} 0(male) = 583 mg/kg bw LD_{50} (female) = 688 mg/kg bw
Reliability	1
Acceptability	acceptable
Remarks	Study is referred to as Report No. T 4025025 which should be T 3025123
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1-1. Acute Oral Toxicity

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
100	0/10	n/a	No abnormal signs during the observation period.
160[a]	0/5	n/a	Slight apathy observed in male (only sex tested) mice beginning shortly after administration and lasting 2 hours – 3 days.
250	1/10	3.5 h	Apathy and tremor observed in mice of both sexes, prostration on side observed in female mice. Apathy resolved within 1 day, other symptoms resolved within 4.3 hours. No pathological findings.
500	2/10	4.9 – 24 h	Apathy and tremor observed in mice of both sexes, prostration on side observed in male mice. Apathy resolved within 2 days, other symptoms resolved within 1 day. One animal with lung distended, one animal with patch lung and dark mucous in glandular stomach.
630	6/10	24 h	Apathy and tremor seen in female mice. Apathy, tremor, spasmodic tremor, prostration on side, dyspnoea, and bristling coat seen in males. All symptoms resolved within 3 days. One male with slightly patch liver, one male with slightly patch lung and liver, one male with distended stomach filled with dark mucous and yellowish-urine filled bladder, one female with slightly patchy liver, one female with slightly patchy liver and distended lung.
710	5/10	3.7 – 24 h	Apathy and tremor seen in both sexes. In female mice, spasmodic tremor and prostration on side also observed. All symptoms resolved within 3 days. One male with distended lung, one male with slightly pale, patchy liver, one male with slightly pale patchy liver and slightly patchy lung; one female with slightly patch liver and dark mucous in glandular stomach, one female with pale patchy liver.
1000	8/10	1.5 – 24 h	Apathy, tremor, spasmodic tremor, prostration on the side observed in both sexes. Bristling coat observed in females. All symptoms resolved within 2 days. Four males with distended lungs, one with slightly pale liver; one female with lung distended and slightly pale liver, one female with lung distended, one female with liver, spleen and kidneys slightly pale and ulceroid foci in stomach, one female with slightly patchy liver.
1000[a,b]	2/5	24 h	Apathy, tremor, spasmodic tremor and bristling coat observed in males (only sex tested). All symptoms resolved within 5 days. One male with slightly pale patch liver, pale spleen, dark mucous in glandular stomach, one male with dark mucous in stomach, distended intestinal tract.

Continued

Table A6.1-1. continued

1600[a]	5/5	1.5 – 24 h	Apathy, tremor, spasmodic tremor and prostration on side observed in males (only sex tested). All symptoms resolved within 1 day. Four animals with distended lung, two with slight liver lobulation, three with pale livers, one with pale kidney and stomach filled with dark mucous.
5000[b]	9/10	1 – 4.7 h	Tremor, spasmodic tremor and prostration on side observed in both sexes. All symptoms resolved within 1 day. Nine animals with distended lung, four males and three females with liver lobulation, one female with pale liver.
LD_{50} value	Male: 583 mg/kg bw Female: 688 mg/kg bw		

[a]Males only. [b]Dose not used for calculation of LD_{50}.

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Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/
Annex Point VI.6.1.2

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		1 REFERENCE
1.1	**Reference**	[redacted] (1999). NAK 4455 (c.n.: Transfluthrin (prop)). Study for acute dermal toxicity in mice [redacted] [redacted] Report No. T 5025026 [BES Ref: MO-03-009373] Report date: 12 February 1999 Unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience
1.2.2	Companies with letters of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes OECD 402 (1987) US EPA OPPTS § 870.1200 (1998) Directive 67/548/EEC Annex V, B.3 (1992)
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3 MATERIALS AND METHODS
3.1	**Test material**	NAK 4455 (transfluthrin)
3.1.1	Lot/Batch number	816779504
3.1.2	Specification	As given in sections 2 and 3
3.1.2.1	Description	Clear, colourless liquid
3.1.2.2	Purity	95.8%
3.1.2.3	Stability	Test substance was stored at room temperature and kept stable throughout the study.

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Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/ Annex Point VI.6.1.2

3.2	**Test Animals**		
3.2.1	Species	Mouse	
3.2.2	Strain	NMRI:WU	
3.2.3	Source	[redacted]	
3.2.4	Sex	Male and female	
3.2.5	Age/weight at study initiation	8 weeks Weight range of 36 – 41 g (males) and 25 – 30 g (females)	
3.2.6	Number of animals per group	5 mice/sex/group	
3.2.7	Control animals	No	
3.3	**Administration/ Exposure**	Dermal	
3.3.1	Post exposure period	14 days	
		Dermal	
3.3.2	Area covered	5 cm^2 site	
3.3.3	Occlusion	Occlusive	
3.3.4	Vehicle	None, undiluted test substance was used	
3.3.5	Concentration in vehicle	2000 or 4000 mg/kg	X
3.3.6	Total volume applied	Not relevant (undiluted)	X
3.3.7	Duration of exposure	24 hr	
3.3.8	Removal of test substance	Treated area was cleaned with soap and water.	
3.3.9	Controls	None	
3.3	**Examinations**	Clinical observations, necropsy, body weight	
3.4	**Method of determination of LD_{50}**	No statistical analysis was necessary for determining the LD_{50}.	
3.5	**Further remarks**	None	

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Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/
Annex Point VI.6.1.2

4 RESULTS AND DISCUSSION

4.1	**Clinical signs**	At 2000 mg/kg bw and above, temporary tremor was observed in both sexes. In one male, piloerection, decreased motility and reactivity, laboured breathing and narrowed palpebral fissure were observed up to day 15. Additionally, this animal had decreased body weight on day 8. This animal was later discovered to have a non treatment related adhesion in the fatty tissue of the abdominal cavity. At 4000 mg/kg bw, motility was affected and temporary convulsions occurred. The symptoms began on day 2 and continued up to day 7 of the study.
4.2	**Pathology**	No treatment-related abnormal findings in animals sacrificed at end of study. In animals that died during the study, the following observations were made: autolysis; discoloration or pale liver, spleen, kidney.
4.3	**Other**	One animal of each sex died in the 4000 mg/kg bw group. Treatment area was reddened in all animals in the 4000 mg/kg bw group.
4.4	**LD_{50}**	The LD_{50} was > 4000 mg/kg bw (highest dose tested) for both males and females.

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Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/ Annex Point VI.6.1.2

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Test material is NAK 4455, Batch no. 816779504, and has a purity of 95.8%. The study was carried out against the following guidelines: OECD Guideline for Testing of Chemicals, Section 4: Health Effects, No. 402 – "Acute Dermal Toxicity", adopted 24 February 1987; Health Effects Test Guidelines (OPPTS § 870.1200), Acute Dermal Toxicity (U.S. EPA, EPA 712-C-98-192, August, 1998); Annex V, Part B.3 (acute toxicity [dermal]) to Directive 67/548/EEC of the Council of the European Communities as amended by Directive 92/69/EEC (1992).

A single dose application was made to a 5 cm^2 area of body surface on the clipped dorsum of the mouse. The undiluted test substance was applied onto the gauze layer of an airtight coated bandage and secured using stretch tape and removed 24 hours after application. The test site was washed with soap and water to remove any residual test material. Five animals of each sex were dosed with either 2000 or 4000 mg test material/kg bw.

Clinical signs and bodyweight were recorded for 14 days post administration. All animals were subjected to macroscopic examination at death.

Based on the result that the lethal dose was more than 4000 mg/kg bw (the highest concentration tested) an LD_{50} value was not calculated.

5.2 Results and discussion

At 2000 mg/kg bw and above, temporary tremor was observed in both sexes. At 4000 mg/kg bw, motility was affected and temporary convulsions occurred. The symptoms began on day 2 and continued up to day 7 of the study.

No treatment related effects on body weight gain were observed.

Two animals (one each male and female) in the high dose group died during the study. The male had autolysis, the female discoloured and pale liver, spleen or kidney. No treatment related effects were seen in animals terminated at end of study.

Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is deemed necessary.

5.3 Conclusion

5.3.1 Reliability 1

5.3.2 Deficiencies No

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Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/
Annex Point VI.6.1.2

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26-01-2007
Materials and Methods	information at 3.3.5 and 3.3.6 should be swapped. 3.3.5 Concentration in vehicle: not relevant (undiluted) 3.3.6 Total volume applied: 2000 or 4000 mg/kg bw Otherwise, the version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	applicant's version is adopted; LD_{50} >4000 mg/kg bw (male and female)
Reliability	1
Acceptability	acceptable
Remarks	Study is referred to as Report No. T 5025026 which should be T 9067529
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc. IIIA Acute Toxicity

Acute dermal toxicity in the mouse

SECTION A6.1.2

BPD Data set IIA/
Annex Point VI.6.1.2

Table A6.1.2-1. Acute Dermal Toxicity

Dose [mg/kg bw]	Number of dead / number of investigated	Time of death (range)	Observations
2000	0/10	n/a	No effects seen in two male and two female mice. Temporary tremor was observed in three female and two male mice resolving by day 3. The remaining male mouse had piloerection, decreased motility and reactivity, and laboured breathing from day 8 to day 15, and narrowed palpebral fissure from day 10 to day 15, had reduced body weight gain on day 8, and on necropsy was found to have a large light coloured adhesion of the fatty tissue in the abdomen and an enlarged pale spleen. No other pathological findings were observed.
4000	2/10	3d, 5d	All animals had local reddening of skin, temporary tremors and temporary convulsions. All symptoms were resolved by day 7. In all animals except two females, increased motility followed by decreased motility was observed. In these two females, one displayed decreased followed by increased motility, and the other only displayed increased motility. Changes in motility had resolved by day 8. Two animals (one each male and female) in the high dose group died during the study. The male had autolysis, the female discoloured and pale liver, spleen or kidney. No other pathological findings were observed.
LD_{50} value	> 4000 mg/kg for males and females		

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Acute Toxicity

Acute inhalation toxicity in the rat

SECTION A6.1.3

BPD Data set IIA/
Annex Point VI.6.1.3

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		6 REFERENCE
6.1	**Reference**	[redacted] 1988) NAK 4455 (c.n. benfluthrin, proposed). Study for acute inhalation toxicity to OECD guideline no. 403. [redacted] [redacted] Report No. T 5029572 [BES Ref: MO-03-009218] Report date: 14 October 1988 Unpublished
6.2	**Data protection**	Yes
6.2.1	Data owner	Bayer CropScience
6.2.2	Companies with letters of access	
6.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		7 GUIDELINES AND QUALITY ASSURANCE
7.1	**Guideline study**	Yes OECD 403 (1981) EC B.2 (1984) FIFRA § 81-3 (1984)
7.2	**GLP**	Yes
7.3	**Deviations**	No
		8 MATERIALS AND METHODS
8.1	**Test material**	NAK 4455 (transfluthrin)
8.1.1	Lot/Batch number	250 987 (mixed batch)
8.1.2	Specification	As given in sections 2 and 3
8.1.2.1	Description	Crystalline mass, brownish, solid
8.1.2.2	Purity	94.5%
8.1.2.3	Stability	Test substance was stored in at room temperature with light excluded; stability was ensured for period of study

Doc. IIIA Acute Toxicity

Acute inhalation toxicity in the rat

SECTION A6.1.3

BPD Data set IIA/ Annex Point VI.6.1.3

8.2	**Test Animals**	
8.2.1	Species	Rat
8.2.2	Strain	Bor: WISW (SPF-Cpb)
8.2.3	Source	[redacted]
8.2.4	Sex	Male and female
8.2.5	Age/weight at study initiation	2 – 3 months Weight range of 160 – 200 g
8.2.6	Number of animals per group	5 mice/sex/group
8.2.7	Control animals	Yes, but not concurrent
8.3	**Administration/ Exposure**	Inhalation
8.3.1	Postexposure period	14 days
		Inhalation
8.3.2	Concentrations	Nominal concentration 5000 [mg/m³] Analytical concentration 513 [mg/m³]
8.3.3	Particle size	MMAD (mass median aerodynamic diameter) 1.44 [µm] ± GSD (geometric standard deviation) 1.42 [µm]
8.3.4	Type or preparation of particles	The aerosol was sprayed under dynamic conditions, using a nozzle and compressed air into a cylindrical inhalation chamber with baffle chamber. The conditions of generation of the aerosol ensure about 30 air exchanges per hour. Air flow was monitored continuously. The air samples for analytical determination and particle distribution were taken in the rats immediate inhalation area. NAK 4455 concentration was analysed by GC. Particle distribution was analysed with an aerodynamic particle size with Laser Velocimeter.
8.3.5	Type of exposure	Nose/head only
8.3.6	Vehicle	Polyethylene glycol E 400:ethanol (1:1)
8.3.7	Concentration in vehicle	25% dilution (maximum achievable concentration)
8.3.8	Duration of exposure	4 h
8.3.9	Controls	Vehicle exposure (not concurrent)
8.4	**Examinations**	Clinical observations, necropsy, body weight, reflexes
8.5	**Method of determination of LD_{50}**	Rosiello, Essigmann and Wogan as modified by Pauluhn based on Bliss, Litchfield and Wilcoxon, Finney, Weil, Thompson, Miller and Tainter
8.6	**Further remarks**	None

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Acute Toxicity

Acute inhalation toxicity in the rat

SECTION A6.1.3

BPD Data set IIA/
Annex Point VI.6.1.3

9 RESULTS AND DISCUSSION

9.1	**Clinical signs**	No symptoms were seen in the male rats. Female rats showed slight tremor (resolving within 5 minutes) immediately after exposure. No effect was seen on body weight. No effect was seen on reflexes. No mortality was seen in study.
9.2	**Pathology**	No abnormal findings were seen in animals sacrificed at end of study.
9.3	**Other**	No other significant effects
9.4	**LD_{50}**	LD_{50} (male): > 513 mg/m^3 LD_{50} (female): > 513 mg/m^3

10 APPLICANT'S SUMMARY AND CONCLUSION

10.1	**Materials and methods**	Test material is NAK 4455, mixed batch no. 250 987, and has a purity of 94.5%. The study was carried out against the following guidelines: OECD Guideline for Testing of Chemicals, Section 4: health Effects, No. 403 – "Acute Inhalation Toxicity", adopted 12th May 1981, and EC B.2 (1984) and FIFRA § 81-3 Acute inhalation toxicity (1984). Groups of 5 male and female Wistar rats were head/nose exposed to NAK 4455 for 4 hours in air at doses (analytical concentrations) of 513 mg/m^3. The MMAD was 1.44 µm, the geometric standard deviation was approximately 1.42 µm, making the particles readily inhalable. All rats were sacrificed at the end of observation period. Clinical signs and bodyweight were recorded for 14 days post administration. All animals were subjected to macroscopic examination at death.
10.2	**Results and discussion**	Clinical signs included slight tremor in exposed female animals—resolving within 5 minutes. No other treatment related effects were seen. The LD_{50} was calculated to be > 513 mg/m^3 for males and females. Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is necessary.
10.3	**Conclusion**	
10.3.1	Reliability	1
10.3.2	Deficiencies	No

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Acute Toxicity

Acute inhalation toxicity in the rat

SECTION A6.1.3

BPD Data set IIA/
Annex Point VI.6.1.3

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	26-01-2007
Materials and Methods	The version of the applicant is acceptable 22-3-2011: 0.513 mg/l was the highest achievable concentration.
Results and discussion	The version of the applicant is adopted
Conclusion	The version of the applicant is adopted; LC_{50} > 513 mg/m^3 (male and female)
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.1.3-1. Acute Inhalation Toxicity

Dose [mg/m^3]	Number of dead / number of investigated	Time of death (range)	Observations
0	0/20	n/a	No abnormal signs during the observation period.
500[a]	0/10	n/a	Slight tremor observed in all females. Resolved after 5 minutes. No effects in male animals. No pathological findings.
LD_{50} value	Male: > 513 mg/m^3 Female: > 513 mg/m^3		

[a]Males only. [b]Dose not used for calculation of LD_{50}.

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Acute Dermal Irritation

Skin irritation study in the rabbit

SECTION A6.1.4/01

BPD Data set IIA/
Annex Point VI.6.1.4

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		11 REFERENCE
11.1	**Reference**	[redacted] (1987); NAK 4455.
		Study for irritant/corrosive potential for skin and eye (rabbit) to OECD guideline nos. 404 and 405; [redacted]
		[redacted] Report No. 5023406 [BES Ref: MOS-03-010099]
		Report date: 20 May 1987
		Unpublished
11.2	**Data protection**	Yes
11.2.1	Data owner	Bayer CropScience
11.2.2	Companies with letters of access	
11.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		12 GUIDELINES AND QUALITY ASSURANCE
12.1	**Guideline study**	Yes
		OECD 404 (1981)
		EC B.4. Acute toxicity (skin irritation) 1984
		FIFRA Guideline No. 81.5 (1984) Primary dermal irritation
12.2	**GLP**	Yes
12.3	**Deviations**	No
		13 MATERIALS AND METHODS
13.1	**Test material**	NAK 4455 (transfluthrin)
13.1.1	Lot/Batch number	NAK 4455-III-4733 of June 5, 1986
13.1.2	Specification	As given in sections 2 and 3
13.1.2.1	Description	Colourless liquid
13.1.2.2	Purity	95.0%
13.1.2.3	Stability	Guaranteed stability to December 26, 1986

Doc. IIIA Acute Dermal Irritation

Skin irritation study in the rabbit

SECTION A6.1.4/01

BPD Data set IIA/
Annex Point VI.6.1.4

13.2	**Test Animals**	
13.2.1	Species	Rabbit
13.2.2	Strain	HC:NZW
13.2.3	Source	[redacted]
13.2.4	Sex	Female
13.2.5	Age/weight at study initiation	Adult Weight range of 3.0 – 3.7 kg
13.2.6	Number of animals per group	Three
13.2.7	Control animals	No
13.3	**Administration/ Exposure**	Dermal
13.3.1	Application	
13.3.1.1	Preparation of test substance	Test substance was used as delivered.
13.3.1.2	Test site and Preparation of Test Site	Test material was place on a "Hypoallergen" dressing and applied to a 6 cm2 area of clipped intact flank. After exposure, exposed skin area was washed with water.
13.3.2	Occlusion	Semiocclusive
13.3.3	Vehicle	None
13.3.4	Concentration in vehicle	Not applicable, no vehicle used
13.3.5	Total volume applied	0.5 ml per animal
13.3.6	Removal of test substance	Water
13.3.7	Duration of exposure	4 h
13.3.8	Postexposure period	1 week
13.3.9	Controls	Opposite flank was treated similarly, but water was used instead of test material.
13.4	**Examinations**	
13.4.1	Clinical signs	No
13.4.2	Dermal examination	Yes

Doc. IIIA Acute Dermal Irritation

Skin irritation study in the rabbit

SECTION A6.1.4/01

BPD Data set IIA/ Annex Point VI.6.1.4

13.4.2.1	Scoring system	Draize
13.4.2.2	Examination time points	60 min, 24 h, 48 h, 72 h and 1 week
13.4.3	Other examinations	None
13.5	**Further remarks**	

14 RESULTS AND DISCUSSION

14.1	**Average score**	
14.1.1	Erythema	Average score for all animals at 24, 48, 72 h was 0.
14.1.2	Oedema	Average score for all animals at 24, 48, 72 h was 0.
14.2	**Reversibility**	Not applicable
14.3	**Other examinations**	None stated
14.4	**Overall result**	Primary irritation score 0.0 (total score of skin reaction over 72 hours)

15 APPLICANT'S SUMMARY AND CONCLUSION

15.1	**Materials and methods**	Test material is NAK 4455, batch no. NAK 4455-III-4733 of June 5, 1986, and has a purity of 95.0%. The study was carried out against the following guidelines: EC B.4. Acute toxicity (skin irritation) 1984; OECD 404 (1981), Acute Dermal Irritancy/Corrosivity, US EPA FIFRA 81.5 (1984) Primary dermal irritation. The test material was applied as a single dose (0.5 mL) to a "hypoallergen" dressing (treated area ~ 6 cm^2); the dressing was applied to the clipped flank of three New Zealand White rabbits. The opposite flank was treated the same way, but water was applied to the dressing. Dressing were fastened with semiocclusive elastic adhesive tape and removed after 4 hours. Treated areas were washed with water. Dermal reactions were observed 1, 24, 48, 72 hours and 1 week after removal of the dressings and scored in accordance with the Draize scale.
15.2	**Results and discussion**	No erythema or oedema was observed at any timepoint. Based on the results of this study, the General Classification and Labelling requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no risk phrase is required for transfluthrin in respect of its irritancy to skin.
15.3	**Conclusion**	
15.3.1	Reliability	1
15.3.2	Deficiencies	No

Doc. IIIA Acute Dermal Irritation

Skin irritation study in the rabbit

SECTION A6.1.4/01

BPD Data set IIA/
Annex Point VI.6.1.4

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05-02-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	The version of the applicant is adopted
Conclusion	Transfluthrin is not a skin irritant
Reliability	1
Acceptability	acceptable
Remarks	-
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Section 6.1.4/02
BPD Data set IIA/
Annex Point VI.6.1.4

Acute Eye Irritation

Eye irritation study in the rabbit

Official use only

16 REFERENCE

16.1	**Reference**	[redacted] (1987). NAK 4455. Study for irritant/corrosive potential for skin and eye (rabbit) to OECD guideline nos. 404 and 405. [redacted]. [redacted] Report No. T 5023406 [BES Ref: MO-03-010099] Report date: 20 May 1987 Unpublished
16.2	**Data protection**	Yes
16.2.1	Data owner	Bayer CropScience
16.2.2	Companies with letters of access	
16.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

17 GUIDELINES AND QUALITY ASSURANCE

17.1	**Guideline study**	Yes OECD 405 (1981) EC B.5. Acute toxicity (eye irritation) 1984 FIFRA Guideline No. 81.4 (1984) Primary eye irritation
17.2	**GLP**	Yes
17.3	**Deviations**	No

18 MATERIALS AND METHODS

18.1	**Test material**	NAK 4455 (transfluthrin)
18.1.1	Lot/Batch number	NAK 4455-III-4733 of June 5, 1986
18.1.2	Specification	As given in sections 2 and 3
18.1.2.1	Description	Colourless liquid
18.1.2.2	Purity	95.0%
18.1.2.3	Stability	Guaranteed stability to December 26, 1986

Doc. IIIA
Section 6.1.4/02

Acute Eye Irritation

Eye irritation study in the rabbit

BPD Data set IIA/
Annex Point VI.6.1.4

18.2	**Test Animals**	
18.2.1	Species	Rabbit
18.2.2	Strain	HC:NZW
18.2.3	Source	[redacted]
18.2.4	Sex	Female
18.2.5	Age/weight at study initiation	Adult Weight range of 3.0 – 3.7 kg
18.2.6	Number of animals per group	Three
18.2.7	Control animals	No
18.3	**Administration/ Exposure**	
18.3.1	Preparation of test substance	Test substance was used as delivered
18.3.2	Amount of active substance instilled	0.1 ml
18.3.3	Exposure period	24h (Single instillation, eye was rinsed with physiological saline after exposure period)
18.3.4	Postexposure period	1 week
18.4	**Examinations**	
18.4.1	Ophthalmoscopic examination	Yes, treated eyes were examined for local reaction visually and with the aid of optical instruments. In addition, dacryorrhoea (discharge) was assessed. After observation at 24 h (and later in case of positive findings), 1% fluorescein stain was use to further examine the eye.
18.4.1.1	Scoring system	Draize
18.4.1.2	Examination time points	60min, 24h, 48h, 72h and 1 week
18.4.2	Other investigations	None
18.5	**Further remarks**	None

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Section 6.1.4/02
BPD Data set IIA/ Annex Point VI.6.1.4

Acute Eye Irritation

Eye irritation study in the rabbit

		19 RESULTS AND DISCUSSION	
19.1	**Clinical signs**	No abnormality was observed in any animals during the experimental period.	
19.2	**Average score**		
19.2.1	Cornea	Average scores for all animals at 24, 48, 72 h was 0	
19.2.2	Iris	Average scores for all animals at 24, 48, 72 h was 0	
19.2.3	Conjunctiva		
19.2.3.1	Redness	Average scores for all animals at 24, 48, 72 h was 0	
19.2.3.2	Chemosis	Average scores for all animals at 24, 48, 72 h were 0.3, 0, 0	
19.3	**Reversibility**	Yes Chemosis was completely reversible within 24 hr	
19.4	**Other**	None	
19.5	**Overall result**	The maximum mean total score (MMTS) was 0, 72 hrs after application.	
		20 APPLICANT'S SUMMARY AND CONCLUSION	
20.1	**Materials and methods**	Test material is NAK 4455, batch no. NAK 4455-III-4733 of June 5, 1986, and has a purity of 95.0%. The study was carried out against the following guidelines: EC B.5. Acute toxicity (eye irritation) 1984; OECD 405 (1981), Acute Eye Irritancy/Corrosivity, US EPA FIFRA 81.4 (1984) Primary eye irritation. The test material was instilled (0.1 mL) into one conjunctiva sac of the lower eyelid of each of the three New Zealand White rabbits. The eyelids were closed for one second after application in order to prevent loss of the test material. The remaining eye of each animal was left untreated as a control. Twenty-four hours after instillation, the treated eye was rinsed with physiological saline. Local reactions were observed 1, 24, 48, 72 hours and 1 week after application and scored in accordance with the Draize scale.	
20.2	**Results and discussion**	Slight chemosis was observed in 1 animal at 24 hours. No other reactions were seen at 24, 48 or 72 hours. Based on these results, the maximum mean total score (MMTS) was 0, 72 hrs after application. Based on the results of this study, the General Classification and Labelling requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no risk phrase is required for transfluthrin in respect of its irritancy to the eye.	X
20.3	**Conclusion**		X
20.3.1	Reliability	1	
20.3.2	Deficiencies	No	

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Section 6.1.4/02

BPD Data set IIA/ Annex Point VI.6.1.4

Acute Eye Irritation

Eye irritation study in the rabbit

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05-02-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	5.2 Second paragraph should be moved to 5.3 (conclusion) Otherwise the version of the applicant is adopted
Conclusion	Transfluthrin is not an eye irritant
Reliability	1
Acceptability	acceptable
Remarks	At t=1h considerable chemosis, redness and dacryorrhoea are observed.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc. IIIA
Section 6.1.4/02

BPD Data set IIA/
Annex Point VI.6.1.4

Acute Eye Irritation

Eye irritation study in the rabbit

Table A6.1.4 (02) -1. Eye irritation study

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
60 min	0	0	1	2.3
24 h	0	0	0	0.3
48 h	0	0	0	0
72 h	0	0	0	0
Average 24h, 48h, 72h	0	0	0	0.1
Area effected	n/a	n/a	n/a	n/a
Maximum average score (including area affected, max 110)	n/a	n/a	n/a	n/a
Reversibility*	n/a	n/a	n/a	c
Average time for reversion	n/a	n/a	n/a	<24h
Give method of calculation maximum average score. * *c : completely reversible* *n c : not completely reversible* *n : not reversible*	n/a	n/a	n/a	*n/a*

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SECTION A6.1.5

BPD Data set IIA/ Annex Point VI.6.1.5

Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

Official use only

	21 REFERENCE	
21.1	**Reference**	[redacted] (1989). NAK 4455 techn. Study for skin-sensitising effect on guinea pigs (Buehler test). [redacted]. [redacted] Report No. T 6029915 [BES Ref: MO-03-006776] Report date: April 14, 1989 Unpublished
21.2	**Data protection**	Yes
21.2.1	Data owner	Bayer CropScience
21.2.2	Companies with letters of access	
21.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	22 GUIDELINES AND QUALITY ASSURANCE	
22.1	**Guideline study**	Yes OECD 406 (1981) FIFRA Guideline No. 81.6 (1984) EC Guideline 84/449 Acute toxicity—Sensitisation of skin (1984)
22.2	**GLP**	Yes
22.3	**Deviations**	No
	23 MATERIALS AND METHODS	
23.1	**Test material**	NAK 4455 (transfluthrin)
23.1.1	Lot/Batch number	250987
23.1.2	Specification	As given in sections 2 and 3
23.1.2.1	Description	Solid brown mass (at room temp), after melting (at 50°C): brown-yellow clear liquid
23.1.2.2	Purity	95.0%
23.1.2.3	Stability	Stable until October 27 1988
23.1.2.4	Preparation of test substance for application	for induction: used as delivered (melted) for challenge: used as delivered (melted)
23.1.2.5	Pretest performed	No

Doc. IIIA Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

SECTION A6.1.5

BPD Data set IIA/ Annex Point VI.6.1.5

	on irritant effects	
23.2	**Test Animals**	
23.2.1	Species	Guinea pigs
23.2.2	Strain	DHPW (SPF)
23.2.3	Source	[redacted]
23.2.4	Sex	Male
23.2.5	Age/weight at study initiation	5 – 7 weeks Body weight range of 348 to 428 g.
23.2.6	Number of animals per group	12 animals per group
23.2.7	Control animals	Yes, 12 for first challenge, 12 kept ready for possible second challenge
23.3	**Administration/ Exposure**	Non-Adjuvant
23.3.1	Induction schedule	Day 0 – day –7 – day 14
23.3.2	Way of Induction	Topical Occlusive
23.3.3	Concentrations used for induction	0.5 ml undiluted test substance was applied to a hypoallergenic dressing and the left flank of the animal. A similar dressing without test compound was applied to the animals in the control group.
23.3.4	Concentration Freunds Complete Adjuvant (FCA)	Not applicable
23.3.5	Challenge schedule	The challenge phase was carried out on day 28 (4 weeks after first induction). Dermal responses were assessed 24, 48 and 72 hours after application.
23.3.6	Concentrations used for challenge	0.5 ml undiluted test compound
23.3.7	Rechallenge	No
23.3.8	Scoring schedule	24h, 48h, 72h after challenge
23.3.9	Removal of the test substance	Test substance was removed after 24 hours; any test material residue was removed with sterile physiological saline solution.
23.3.10	Positive control substance	Not stated, however the strain's sensitivity for sensitisation tests is checked at regular intervals and documented.
23.4	**Examinations**	
23.4.1	Pilot study	No
23.5	**Further remarks**	

Doc. IIIA Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

SECTION A6.1.5

BPD Data set IIA/
Annex Point VI.6.1.5

24 RESULTS AND DISCUSSION

24.1	**Results of pilot studies**	Not applicable
24.2	**Results of test**	
24.2.1	24h after challenge	No skin reactions were observed in treated (0/12) or control (0/12) animals.
24.2.2	48h after challenge	No skin reactions were observed in treated (0/12) or control (0/12) animals.
24.2.3	Other findings	Body weights were measured before the start and at the end of the study. The body weights of all animals increased normally during the experiment. No clinical symptoms were noted.
24.3	**Overall result**	Based on the results obtained NAK 4455 was considered to have no sensitising potential under the conditions of this test.

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SECTION A6.1.5

BPD Data set IIA/ Annex Point VI.6.1.5

Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

25 APPLICANT'S SUMMARY AND CONCLUSION

25.1 Materials and methods

Test material is NAK 4455, batch no. 250987, and has a purity of 95%. The study was carried out against the following guidelines: OECD 406 Skin Sensitisation (1981), FIFRA Guideline No. 81.6 (1984), EC Guideline 84/449 Acute toxicity—Sensitisation of skin (1984).

Test groups consisted of: NAK 4455 sensitised group of 12 males, 12 control males for test material.

Induction phase: Induction occurred three times (every 7 days). 0.5 ml undiluted test substance was applied to a hypoallergenic dressing and applied to the shorn left flank of the animal and fastened with an occlusive dressing. The animals in the control group were treated the same way, however no compound was applied to the dressing. After 6 hours, the dressing was removed and residue washed off with physiological saline.

Challenge phase: Challenge occurred 4 weeks after 1st induction. 0.5 ml undiluted test substance was applied to a hypoallergenic dressing and applied to the shorn left flank of the induced and control animals and fastened with an occlusive dressing. A dressing without test material was applied to the right flank of all animals as a control. After 6 hours, the dressing was removed and residue washed off with physiological saline.

Dermal response was assessed 24 h after removal of induction dressing and 24, 48 and 72 h after removal of challenge dressing. Current guidelines call for 20 animals per group for a Buehler test and concurrent positive controls. The strain used for this test is regularly tested for sensitivity with positive controls, thus the lack of concurrent positive control is not expected to affect results. Due to the complete lack of irritation or sensitisation potential of this compound, the use of 12 animals rather than 20, is not expected to affect the results.

25.2 Results and discussion

The results from the challenge procedure with NAK 4455 indicated that no animals gave a positive response indicative of delayed contact hypersensitivity. The sensitization rate was estimated to be 0% (positive animals/all tested animals = 0/20).

Based on the results of this study, the General Classification and Labelling requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no risk phrase is required for transfluthrin in respect of its skin sensitizing potential.

25.3 Conclusion

25.3.1 Reliability: 2

25.3.2 Deficiencies: Yes

Current guidelines call for 20 animals per group for a Buehler test and concurrent positive controls. The strain used for this test is regularly tested for sensitivity with positive controls, thus the lack of concurrent positive control is not expected to affect results. Due to the complete lack of irritation or sensitisation potential of this compound, the use of

Doc. IIIA

Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

SECTION A6.1.5

BPD Data set IIA/ Annex Point VI.6.1.5

12 animals rather than 20, is not expected to affect the results.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	05-02-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	The version of the applicant is adopted
Conclusion	Although the study does not comply with the OECD 406 data requirements, the 12 animals do not show signs of sensitisation. In view of the negative GMPT study (considered not a key study by the applicant) it is concluded that transfluthrin is not a skin irritant.
Reliability	2
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc. IIIA Skin sensitisation

Skin sensitisation in the Guinea pig (Buehler Test)

SECTION A6.1.5

BPD Data set IIA/
Annex Point VI.6.1.5

Table A6.1.5-1. Skin sensitisation test

Inductions	Buehler test	Observations/Remarks
	day of treatment	
Induction 1	day 0	0.5 ml of undiluted NAK 4455
Induction 2	7	0.5 ml of undiluted NAK 4455
Induction 3	14	0.5 ml of undiluted NAK 4455
challenge	28	0.5 ml of undiluted NAK 4455
scoring 1	24 h	No skin reaction in all test and control animals.
scoring 2	48 h	No skin reaction in all test and control animals.

Table A6.1.5-2. Result of skin sensitisation test

	Number of animals with signs of allergic reactions / number of animals in group		
	Negative control	**Test group**	**Positive control**
scored after 24h	0 / 12	0 / 12	n/a
scored after 48h	0 / 12	0 / 12	n/a

n/a: not available

Doc. IIIA **SECTION 6.2** **BPD Data set IIA/ Annex Point IIIA.6.2**	**Dermal absorption assessment**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	**The Technical Notes for Guidance indicate that percutaneous absorption should be assessed during toxicokinetic studies. An oral study that fully meets the criteria of EC Guidance B.36. Toxicokinetics is summarized in IIIA6.2. That study indicates that a majority of ingested transfluthrin becomes quickly available to the systemic circulation as seen by excretion in urine at 8 and 24 hours. As detailed below, physico-chemical properties of the pyrethroids do not suggest that dermal absorption is significant, and the 10% default dermal penetration value is appropriate for risk assessment. Additionally, section IIIA6.1.2 in this dossier indicates an LD_{50} of >4000 mg/kg for acute dermal toxicity in mice, compared to the acute oral LD_{50} of 583 (male) and 688 (female) in mice, supporting a low dermal penetration value. Given current knowledge that dermal absorption is not a likely route for significant toxicity, and that a fully compliant oral study is available, a specific dermal absorption study to track toxicokinetics is not an appropriate use of animals.** Physico-chemical properties: **Chemicals fulfilling both criteria of molecular weight (MW) >500 and log P_{ow} (lipid solubility) < –1 to > 4 are accepted to have a dermal penetration rate of 10% or less. Transfluthrin has MW 371 and log P_{ow} 5.4; values which (in common with most pyrethroids) are close to the MW criterion and well beyond the P_{ow} criterion. These physico-chemical values strongly suggest dermal penetration substantially less than 10%, as is seen for other pyrethroids (Table 1) which approach but do not exceed the MW criterion. Comparison is particularly valid with cypermethrin (MW 416, log P_{ow} 5.5, dermal penetration in humans 1.8%); or permethrin (MW 391, log K_{ow} 6.1, dermal penetration in humans 2%)** **Pyrethroids, including transfluthrin, are derivatives of permithrinic acid; there is a strong read-across in basic chemical characteristics (on which dermal penetration largely depends).** Comparison with other Pyrethroids **Publicly available data reporting dermal absorption of pyrethroids show consistently low values. Ray (2001)[1] states that in humans, the bioavailability of dermal pyrethroids is about 1%. This statement**	

[1] Ray DE (2001) "Pyrethroid Insecticides: Mechanisms of Toxicty, Systemic Poisoning Syndromes, Paresthesia, and Therapy" (in) Kreiger R (ed) "Handbook of Pesticide Toxicology", 2nd Edn. p1294. Academic Press: San Diego (2001)

Doc. IIIA SECTION 6.2 BPD Data set IIA/ Annex Point IIIA.6.2	Dermal absorption assessment
	cites Woollen et al (1992)[2] who determined that urinary excretion of a 31 mg dose of cypermethrin as a soy-oil based formulation to the forearm of each of 6 volunteers, was approximately 1.2% of applied dose (compared to 36% of an oral dose). A review of pyrethroid toxicology by US ATSDR (2001)[3] estimated dermal penetration at 0.3% - 1.8%, citing (in addition to Woollen *et al*, 1992) work by Eadsforth *et al* (1988) [4] in which approximately 0.1% of a 25 mg Cypermethrin dermal dose to each of 2 volunteers was recovered as metabolites in urine. Using permethrin as a dermal cream in scabies patients, van der Rhee *et al* (1989) estimated from urinary elimination data, absorption of a 1250 mg dose to be approximately 0.5%. Ross *et al* (2001)[5] report 2% in vivo dermal absorption of permethrin in humans. **Data publicly available for other pyrethroids listed in Annex 1 to EU Directive 91/414, shows all to have comparable molecular weight and particularly log P_{ow}, to transfluthrin. Where specific dermal absorption values are given, these are low; in cases where data are absent, the 10% default value has been accepted..** Further Considerations **Human skin is thicker, and for the great majority of chemicals is less permeable, than rat skin. Available data suggests this to be particularly true for the pyrethroids. Scott and Ramsey (1987)[6] found rat skin to be 20 times more permeable to cypermethrin than was human skin. Ross *et al* (2001) calculate a rat:human ratio of 14 for dermal absorption of permethrin; the rat;human ratio for this pyrethroid was greater than for any of the other 12 chemicals (all non-pyrethroids) for which data was presented. The 91/414 EEC Annex 1 critical endpoints for esfenvalerate shows human skin to be very much more protective than rat skin. These data offer further weight of evidence that the dermal penetration of transfluthrin in humans will be very low, and assumption of a 10% default to be both protective, and consistent with assessment of other pyrethroids.**
Undertaking of intended	

[2] Woollen BH, Marsh JR, Laird WJ, Lesser JE (1992) "The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration". Xenobiotica 22(8) 983-991.

[3] Agency for Toxic Substances and Disease Registry (2003): "Toxicological Profile for Pyrethrins and Pyrethroids" Available at: http://www.atsdr.cdc.gov/toxprofiles/tp155.pdf.

[4] Eadsforth CV, Bragt PC, van Sittert NJ (1988) "Human dose-excretion studies with pyrethroid insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring". Xenobiotica 18(5): 603-14

[5] Ross, JH, Driver, JH, Cochran, RC, Thongsinthusak, T, Krieger, RI (2001) "Could pesticide toxicology studies be more relevant to occupational risk assessment?". Ann. Occup. Hyg. 45(1001):S5-S17.

[6] Scott RC, Ramsey JD (1987) "Comparison of the in vivo and in vitro percutaneous absorption of a lipophilic molecule (cypermethrin, a pyethroid insecticide). J.Invest.Dermatol. 89(2) 142-146.

Doc. IIIA	**Dermal absorption assessment**
SECTION 6.2	
BPD Data set IIA/ Annex Point IIIA.6.2	
data submission [ ]	

Doc. IIIA Section 6.2 Annex Point IIIA.6.2	Dermal absorption assessment	
	Justification for non-submission of data	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ] Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The Technical Notes for Guidance indicate that percutaneous absorption should be assessed during toxicokinetic studies. An oral study that fully meets the criteria of EC Guidance B.36. Toxicokinetics is summarized in IIIA6.2. That study indicates that a majority of ingested transfluthrin becomes quickly available to the systemic circulation as seen by excretion in urine at 8 and 24 hours. As detailed below, physico-chemical properties of the pyrethroids do not suggest that dermal absorption is significant, and the 10% default dermal penetration value is appropriate for risk assessment. Additionally, section IIIA6.1.2 in this dossier indicates an LD_{50} of >4000 mg/kg for acute dermal toxicity in mice, compared to the acute oral LD_{50} of 583 (male) and 688 (female) in mice, supporting a low dermal penetration value. Given current knowledge that dermal absorption is not a likely route for significant toxicity, and that a fully compliant oral study is available, a specific dermal absorption study to track toxicokinetics is not an appropriate use of animals. **Physico-chemical properties:** Chemicals fulfilling both criteria of molecular weight (MW) >500 and log P_{ow} (lipid solubility) < –1 to > 4 are accepted to have a dermal penetration rate of 10% or less. Transfluthrin has MW 371 and log P_{ow} 5.4; values which (in common with most pyrethroids) are close to the MW criterion and well beyond the P_{ow} criterion. These physico-chemical values strongly suggest dermal penetration substantially less than 10%, as is seen for other pyrethroids (Table 1) which approach but do not exceed the MW criterion. Comparison is particularly valid with cypermethrin (MW 416, log P_{ow} 5.5, dermal penetration in humans 1.8%); or permethrin (MW 391, log K_{ow} 6.1, dermal penetration in humans 2%) Pyrethroids, including transfluthrin, are derivatives of permithrinic acid; there is a strong read-across in basic chemical characteristics (on which dermal penetration largely depends). **Comparison with other Pyrethroids** Publicly available data reporting dermal absorption of pyrethroids show consistently low values. Ray (2001)[7] states that in humans, the bioavailability of dermal pyrethroids is about 1%. This statement cites Woollen et al (1992)[8] who determined that urinary excretion of a 31 mg	

[7] Ray DE (2001) "Pyrethroid Insecticides: Mechanisms of Toxicty, Systemic Poisoning Syndromes, Paresthesia, and Therapy" (in) Kreiger R (ed) "Handbook of Pesticide Toxicology", 2nd Edn. p1294. Academic Press: San Diego (2001)

[8] Woollen BH, Marsh JR, Laird WJ, Lesser JE (1992) "The metabolism of cypermethrin in man: differences in urinary metabolite profiles following oral and dermal administration". Xenobiotica 22(8) 983-991.

Doc. IIIA Section 6.2 Annex Point IIIA.6.2	Dermal absorption assessment
	dose of cypermethrin as a soy-oil based formulation to the forearm of each of 6 volunteers, was approximately 1.2% of applied dose (compared to 36% of an oral dose). A review of pyrethroid toxicology by US ATSDR (2001)[9] estimated dermal penetration at 0.3% - 1.8%, citing (in addition to Woollen *et al*, 1992) work by Eadsforth *et al* (1988) [10] in which approximately 0.1% of a 25 mg Cypermethrin dermal dose to each of 2 volunteers was recovered as metabolites in urine. Using permethrin as a dermal cream in scabies patients, van der Rhee *et al* (1989) estimated from urinary elimination data, absorption of a 1250 mg dose to be approximately 0.5%. Ross *et al* (2001)[11] report 2% in vivo dermal absorption of permethrin in humans. Data publicly available for other pyrethroids listed in Annex 1 to EU Directive 91/414 or under BPD review shows all to have comparable molecular weight and particularly log P_{ow}, to transfluthrin. Where specific dermal absorption values are given, these are low; in cases where data are absent, the 10% default value has been accepted. Furthermore tefluthrin, a pyrethroid with a close chemical structure to transfluthrin (see Figure 1), is characterised by a low dermal absorption (0.12%). **Further Considerations** Human skin is thicker, and for the great majority of chemicals is less permeable, than rat skin. Available data suggests this to be particularly true for the pyrethroids. Scott and Ramsey (1987)[12] found rat skin to be 20 times more permeable to cypermethrin than was human skin. Ross *et al* (2001) calculate a rat:human ratio of 14 for dermal absorption of permethrin; the rat:human ratio for this pyrethroid was greater than for any of the other 12 chemicals (all non-pyrethroids) for which data was presented. The 91/414 EEC Annex 1 critical endpoints for esfenvalerate shows human skin to be very much more protective than rat skin. These data offer further weight of evidence that the dermal penetration of transfluthrin in humans will be very low, and assumption of a 10% default to be both protective, and consistent with assessment of other pyrethroids.
Undertaking of intended data submission [ ]	

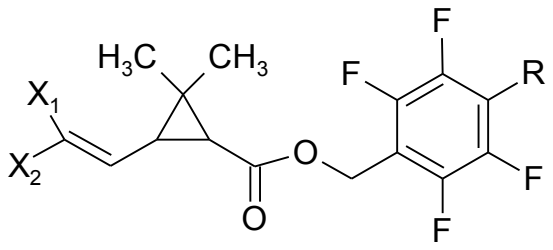
[9] Agency for Toxic Substances and Disease Registry (2003): "Toxicological Profile for Pyrethrins and Pyrethroids" Available at: http://www.atsdr.cdc.gov/toxprofiles/tp155.pdf.

[10] Eadsforth CV, Bragt PC, van Sittert NJ (1988) "Human dose-excretion studies with pyrethroid insecticides cypermethrin and alphacypermethrin: relevance for biological monitoring". Xenobiotica 18(5): 603-14

[11] Ross, JH, Driver, JH, Cochran, RC, Thongsinthusak, T, Krieger, RI (2001) "Could pesticide toxicology studies be more relevant to occupational risk assessment?". Ann. Occup. Hyg. 45(1001):S5-S17.

[12] Scott RC, Ramsey JD (1987) "Comparison of the in vivo and in vitro percutaneous absorption of a lipophilic molecule (cypermethrin, a pyethroid insecticide). J.Invest.Dermatol. 89(2) 142-146.

Figure 1



Compound	X_1	X_2	R
Transfluthrin	**Cl**	**Cl**	**H**
Tefluthrin	**Cl**	**CF_3**	**CH_3**

Table 1: Comparison of dermal penetration characteristics for pyrethroids (particularly where listed in Annex I to EC 91/414 or under BPD review)

Compound name	Dermal absorption	MW	Log P_{ow}	Product[1]	Reference
Alpha-cypermethrin	10% default assumed	416.3	5.5		Review report for the active substance cypermethrin. SANCO/4333/2000 final. 15 February 2005
Beta-cyfluthrin	10% default assumed	434.3	5.9	EC50	Review report for the active substance beta-cyfluthrin. 6841/VI/97-final. 2 December 2002
Cyfluthrin	10% default assumed	434.3	5.9	EC50	Review report for the active substance cyfluthrin. 6843/VI/97-final. 2 December 2002
Deltamethrin	10% default assumed	505.2	4.6	25EC	Review report for the active substance deltamethrin. 6504/VI/99-final. 17 October 2002
Deltamethrin	2%	505.2	4.6	25EC	Assessment Report Deltamethrin. Product-type 18 (Insecticides). May 2011
Esfenvalerate	0.6% (human epidermis) 44% (rat skin)	419.9	6.24	EC	Review report for the active substance esfenvalerate. 6846/VI/97-final. 3 October 2005
Lambda-cyhalothrin	<0.3% (human, in-vivo)	449.9	7.0	5EC	Review report for the active substance lambda-cyhalothrin. 7572/VI/97-final. 25 January 2001
Lambda-cyhalothrin	1% (concentrate,dilution 0.4 g/L)	449.9	7.0	CS	Competent Authority Report. Doc I. lambda-Cyhalothrin. Product type 18: Insecticide. Draft September 2008.
Tefluthrin	0.12%	418.7	6.4	CS	Conclusion on the peer review of the pesticide risk assessment of the active substance tefluthrin EFSA Journal 2010;8(12):1709
Transfluthrin	**Proposed: 10% default**	**371.2**	**6.17**[13]		
Permethrin	2% (human, in-vivo)	391.3	6.1		Ross et al (2001)
Cypermethrin	0.1 –1.8% Human in-vivo	416.3	6.6		Handbook of Pesticide Toxicology, Vol 2 p 1268; Eadsforth (1988); Woollen (1992)

1: Product: type is that which appears to be that used for Annex 1 approval, not confirmed.

[13] KowWin v1.67 estimate

EVALUATION BY COMPETENT AUTHORITIES	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 January 2007 and 21 September 2012
Evaluation of applicant's justification	Considering the available data submitted by the applicant, a default dermal absorption of 10% is justified.
Conclusion	Applicant's justification is acceptable. A dermal absorption of 10% can be adopted for transfluthrin by default.
Remarks	Based on comments from other members states in TMI2011 the dermal absorption percentage of other pyrethroids (including a fluorinated pyrethoid) has been added. The dermal absorption of 10% is still justified.
	COMMENTS FROM OTHER MEMBER STATE (specify)
Date	Give date of comments submitted
Evaluation of applicant's justification	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Remarks	

Doc. IIIA
Section 6.2

BPD Data set IIA/ Annex Point IIA.6.2

Metabolism Studies in Animals – Basic Toxicokinetics in the Rat

Official use only

26 REFERENCE

26.1	**Reference**	[redacted] (1991). Disposition of [Methylene-14C] Benfluthrin (NAK 4455) in Rats. [redacted] Report No. 101310, [redacted] Report No. N 5041801 [BES Ref: MO-03-010378] Report date: 1 October 1991. Unpublished.
26.2	**Data protection**	Yes
26.2.1	Data owner	Bayer CropScience (Mobay Corporation)
26.2.2	Companies with letters of access	
26.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

27 GUIDELINES AND QUALITY ASSURANCE

27.1	**Guideline study**	No, but methods used are comparable to EC Method B.36.
27.2	**GLP**	Yes
27.3	**Deviations**	**Amendments to dosing levels – refer to section 3.3.1.** **There was no direct determination of plasma level or time curve for excretion of radiolabel, but tissue sampling for residues indicated residues to be highest in tissues known to be affected by benfluthrin from other studies and so is consistent. There are no other relevant deviations from the guidelines.**

28 MATERIALS AND METHODS

28.1	**Test material**	As given in section 2, with [methylene-^{14}C, 39 mCi/mmol] for tracking. This study uses the common name benfluthrin when referencing the Bayer identifier NAK4455. Other studies in this dossier use transfluthrin as the common name for the same chemical entity.
28.1.1	Lot/Batch number	Radiolabelled benfluthrin – Lot no. 4813/1 from Bayer AG Non-radioactive benfluthrin – vial no 1008, Bayer reference no. 88032SELB01.
28.1.2	Specification	As given in section 2

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Metabolism Studies in Animals – Basic Toxicokinetics in the Rat

BPD Data set IIA/ Annex Point IIA.6.2

28.1.2.1	Description	Physical appearance not described.
28.1.2.2	Purity	Radiolabelled benfluthrin – radiochemical purity >99% Non-radiolabelled benfluthrin – 97.8% purity
28.1.2.3	Stability	Both radiolabelled and non-radiolabelled benfluthrin were held at 0°C during the dosing period. Under these conditions benfluthrin was found to be stable for at least 30 days.
28.1.2.4	Radiolabelling	Methylene - ^{14}C benfluthrin

28.2 Test Animals

28.2.1	Species	Rat
28.2.2	Strain	Wistar
28.2.3	Source	[redacted]
28.2.4	Sex	Male and female
28.2.5	Age/weight at study initiation	Animals were received weighing from 140 to 200 g bw

28.2.6 Number of animals per group

Study	Sex	Number
PMBE – preliminary material balance experiment	M	3
PEAE – preliminary expired air experiment	M	3
LDE – low dose experiment (0.5 mg/kg)	M and F	5 each
HDE – high dose experiment (5 mg/kg)	M and F	5 each
SHDE – supplemental high dose experiment (200 mg/kg)	M and F	2 each
LDCE – low dose chronic experiment without radiolabelled a.s. (0.5 mg/kg)	M and F	10 each
With one dose of radiolabelled a.s.	M and F	5 each of the rats already treated without radiolabel

28.2.7	Control animals	No

28.3 Administration/ Exposure Oral

28.3.1 Concentration of test substance: 0.5, 5, or 200 mg active substance/kg bw

The study states that the dosing levels were chosen based on conversations with the Toxicology Branch, Health Effects Division of the EPA in June 1990; these discussions resulted in a suggested high dose 100 times greater than the anticipated human level of exposure. The dose known to cause pharmacological symptoms (e,g, 400 mg/kg) might saturate the detoxification systems to be analysed in this study, and therefore the study was designed to be relevant but lower than maximal dose. Described calculations indicated that human exposure levels were only 0.02 mg/kg body weight, insufficient to allow tracking of the radiolabel. The high dose was therefore chosen to be 5 mg/kg, high enough for tracking the radiolabel, but low enough to avoid saturation of detoxification enzyme systems. However, in March 1991 the EPA asked for an increased dose level of 200 mg/kg, which was subsequently included.

28.3.2	Specific activity of test substance	PMBE – 9.935 mCi/mmol, 59420 dpm/µg, a.s. 2.929 mg/ml PEAE – 3.963 mCi/mmol, 23698 dpm/µg, a.s. 2.021 mg a.s./ml LDE – 10.8 µCi/0.5 ml in males, 10.4 µCi/0.5 ml in females (different activity needed to account for different body weights of genders) HDE – 115.4 µCi/0.47 ml in males, 108.8 µCi/0.51 ml in females SHDE – 13.7 µCi/0.43 ml in males, 11.4 µCi/0.36 ml in females LDCE – 15.6 µCi/0.51 ml in males, 10.1 µCi/0.53 ml in females	
28.3.3	Volume applied	The test compound was administered by gavage in approximately 0.5 ml, adjusted for a.s. content	
28.3.4	Vehicle	Polyethylene glycol	
28.3.5	Dosing schedule	Single dose - PMBE, PEAE, LDE, HDE, SHDE 14 daily doses – LDCE 15 daily doses – subset of LDCE; all 10 animals of each gender were dosed with non-radiolabelled a.s. for 14 days, then 5 of each gender received one additional dose of radiolabelled a.s.	
28.3.6	Sample collection	Volatile compounds	Rats were housed in a glass metabolism cage. Air was drawn out of the cages through an ethylene glycol trap and two CO_2 absorption towers containing 2N KOH. 48 hours after dosing aliquots of the ethylene glycol and KOH were radioassayed.
		Urine and faeces	Urine was collected at 8, 24, and 48 hours post-treatment, and faeces were collected at 24 and 48 hours in separate containers. Collection vessels were maintained on dry ice during the experiment. At collection, urine from each rat was radioassayed in triplicate. Faeces were weighed, and homogenized with a methanol wash of the collection vessel. Further methanol washes and the extracted solids were both radioassayed. Urine and faeces also were subjected to HPLC and TLC analysis.
28.3.7		Cage rinse	Upon completion of each experiment, the walls of the cages were rinsed with methanol. The rinses were collected and radioassayed.
28.3.8		Tissues	48 hrs after dosing animals were sacrificed by exsanguination under Halothane. Whole blood, bone (femur), brain, muscle (thigh), skin, fat (renal), heart, kidney, liver, spleen, gonads, thyroid, and GI tract were excised, weighed, and frozen prior to processing and radioassay.
28.3.9	Quantitation of radioactivity	The total percentage of radioactivity was expressed in terms of "administered radioactivity". This allows comparison of results from different experiments and is expressed as percent of dose. The total radioactivity was based on the weight of the sample, or in the case of samples like blood that did not represent the whole body part, it was based on literature estimates of % body weight (e.g. 8% - blood, 11% - fat, 11% - muscle). Residues were calculated as ppm.	

X

28.3.10 Measurement of radioactivity

Aliquots of liquid samples were pipetted into scintillation vials and radioassayed in a liquid scintillation spectrometer.

Solid samples were combusted to $^{14}CO_2$ using an oxidizer. The CO_2 was trapped in an absorbent column, then the column material mixed with scintillation fluid and counted in a liquid scintillation spectrometer.

During HPLC analysis, radioactivity was monitored with a lithium glass scintillation cell determined to be 22% efficient for carbon-14.

29 RESULTS AND DISCUSSION

29.1 Preliminary Studies

PMBE – The preliminary material balance experiment was conducted to determine the recovery of radioactivity and excretion profile following oral administration of ^{14}C-benfluthrin. Three male rats were treated orally with 0.5 mg a.s./kg bw benfluthrin dissolved in 0.5 ml polyethylene glycol. A total of 93% of the administered radioactivity was excreted in the urine and faeces within 48 hours after dosing. The excretion ratio was 5.6 (urine) to 1 (faeces), indicating significant uptake of the chemical from the gastrointestinal tract into the general circulation. 96% of administered radioactivity was recovered in all samples combined (urine, faeces, tissues, carcass, and cage wash).

PEAE – The preliminary expired air experiment was conducted to determine if volatile radioactive products would be expired following administration of ^{14}C-benfluthrin dissolved in 0.5 ml polyethylene glycol. Three male rats were treated orally with 0.5 mg a.s./kg bw benfluthrin. <1% of radioactivity detected was expired as a volatile organic product or as $^{14}CO_2$. On the basis of these results, benfluthrin is considered to be resistant to metabolism to a volatile product. Later experiments were not monitored for volatile radioactivity.

X

29.2 Full experimental studies

LDE – The low dose experiment consisted of five rats of each sex given one oral dose of 0.5 mg a.s./kg bw of ^{14}C-benfluthrin. The gavage dose was administered in a total volume of 0.5 ml polyethylene glycol.

HDE - The high dose experiment consisted of five rats of each sex given one oral dose of 5 mg a.s./kg bw of ^{14}C-benfluthrin (108.8-115.4 μCi). The gavage dose was administered in a total volume of approximately 0.5 ml polyethylene glycol.

SHDE – The supplemental high dose experiment consisted of two rats of each sex given one oral 0.5 ml gavage dose of 200 mg a.s./kg bw of benfluthrin, mixed radiolabelled and non-radiolabelled.

LDCE – The low dose chronic experiment consisted of 10 rats of each sex dosed orally for 14 consecutive days with 0.5 mg a.s./kg bw non-radioactive benfluthrin. On day 15, five of the ten rats of each sex were additionally treated with one dose of radiolabelled benfluthrin at a dose of 0.5 mg a.s./kg bw and tracked as in the single dose experiments.

29.3	**Disposition and recovery of radioactivity (^{14}C-benfluthrin)**	Excretion of radioactivity was rapid in all experiments (LDE, HDE, SDHE, LDCE). At 8 hours, 34-47% of administered radioactivity was recovered in the urine; at 24 hours 73-86% had been recovered, and 74-90% had been excreted in the urine by 48 hours. Within 48 hours 8-24% of administered radioactivity had been recovered in the faeces, the majority within 24 hours. No differences were seen between sexes or experimental groups with the exception that the renal to faecal ratio was reduced to 3.1:1 in the SHDE (from 7.8 to 11.2:1 in LDE, LDCE, and HDE). This reduction in renal to faecal ratio was thought to be due to saturation of the rats' enzyme systems and caused more unmetabolized benfluthrin to be excreted in the faeces. Radioactive residue was highest in the liver and kidney for all animals. Additionally, female rat tissues had generally higher residues than males. The tissue with the lowest radioactive residue was the brain. Not more than 1% of administered radioactivity remained in any one tissue 48 hours after dosing in any experiment. A total of 2% or less of the administered radioactivity was found in tissues and carcasses in all experiments. Tissues tested for remaining radioactivity at 48 hours: blood, brain, bone, fat, gonads, heart, kidney, liver, lung, muscle, skin, spleen, thyroid, GI tract, carcass. 1% of administered radioactivity remained in the livers of males of the LDE and HDE tests, and the GI tract of males in the LCDE test. All other tissues retained <1% administered radioactivity.	X
29.4	**Toxic effects, clinical signs**	No effects were described.	
29.5	**Dermal irritation**	No effects were described; this was an oral study.	
29.6	**Metabolites**	There were two major radioactive peaks found in HPLC analysis of the urine. Peak One was identified as tetrafluorobenzoic acid (50-69% of the administered activity), and Peak Two was a glucuronic acid conjugate of tetrafluorobenzyl alcohol (14-43% of administered activity). No differences in the percentage of tetrafluorobenzoic acid and tetrafluorobenzyl glucuronide excreted were noticed between sexes or experimental groups. Several unidentified radioactive components comprising 1-4% of the administered activity were seen in the composite urine samples, but no one component accounted for >1% of the activity. In the faeces, HPLC analysis showed one major peak that co-chromatographed with benfluthrin. Unmetabolized benfluthrin accounted for 1-3% of administered radioactivity in the LDE, HDE, and LDCE, but represented 10-22% in the SHDE. Other radioactive peaks were detected but none accounted for >2% of administered radioactivity.	X

30 APPLICANT'S SUMMARY AND CONCLUSION

30.1 Materials and methods

A preliminary set of experiments indicated that the major route of excretion of ^{14}C-benfluthrin after a single oral gavage dose of 0.5 mg/kg was in the urine within 48 hours. Ten animals of each gender were therefore dosed with 0.5 mg/kg radiolabelled benfluthrin in a low dose oral experiment, ten of each gender with 5.0 mg/kg in a high dose oral experiment, and two of each gender with 200 mg/kg in a supplemental high dose oral experiment. Ten animals of each gender were also given a gavage dose of 0.5 mg/kg for 14 consecutive days, then five of each gender were given an additional dose of radiolabelled benfluthrin. The excretion of the animals was tracked for 48 hours, and the urine, faeces, tissues, and a cage wash were analyzed for radioactivity.

This test is consistent with EC Method B.36, Toxicokinetics. There was no direct determination of plasma level or time curve for excretion of radiolabel, but tissue sampling for residues indicated residues to be highest in tissues known to be affected by benfluthrin from other studies and so is consistent. There are no other relevant deviations from the guidelines.

30.2 Results and discussion

Benfluthrin was rapidly absorbed and metabolized in rats. 48 hours after oral dosing 96-98% of the administered activity was excreted in the urine and faeces. Approximately 1-2% remained in tissues. The major route of excretion was the urine (74-88%), which was similar in each gender and all but the highest dose group. The highest dose group excreted a greater proportion, although not the majority, in the faeces. This is thought to be due to decreased absorption and/or saturation of enzymatic detoxification systems. The major metabolites of benfluthrin are tetrafluorobenzoic acid and the glucuronic acid conjugate of tetrafluorobenzyl alcohol. X

The dose-response information that can be derived from this study is that the highest dose tested, 200 mg/kg, appears capable of saturating the uptake/metabolizing enzyme system in the rat. This is not correlated with clinical signs, and therefore does not represent a LOAEL. All other doses, including single 0.5 mg/kg and 5.0 mg/kg, and multiple 0.5 mg/kg doses (15 doses) did not produce metabolic or excretory effects different from each other. In no case were adverse clinical signs noted.

30.3 Conclusion

30.3.1 Reliability 1

30.3.2 Deficiencies None

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	30 January 2007
Materials and Methods	The version of the applicant is acceptable, with the following correction: **3.3.9** The total radioactivity based on literature estimates of % body weight was stated to be for muscle 50%, not 11% as reported by the applicant.
Results and discussion	**4.1** – The conclusion of this study needs specification. Based on the results from the preliminary expired air experiment, the tetrafluorobenzyl-methylene moiety of benfluthrin is considered to be resistant to metabolism to a volatile product. **4.6** Correction of applicants summary: Peak Two is 14-34% of administered activity (not 14-43%). In addition to applicants summary: In LDE, HDE and LDCE, at least 88% of total administered radioactivity, was identified. **5.2** The conclusion needs specification. The major metabolites of benfluthrin containing the tetrafluorobenzyl-methylene moiety are tetrafluorobenzoic acid and the glucuronic acid conjugate of tetrafluorobenzyl alcohol.
Conclusion	Conclusion of the applicant is acceptable, with the remark that the conclusion only applies to the benzylmethylene moiety of transfluthrin. This study contains no data on the fate of the carboxylic moiety of transfluthrin.
Reliability	1
Acceptability	Acceptable.
Remarks	No plasma levels of radioactivity or time curve for excretion of radiolabel were determined. However, it is obvious that plasma halflife times will be short. In addition, plasma halflifes have been determined in other, non-key studies submitted.

COMMENTS FROM ...

Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Document IIIA Metabolism Studies in Animals – Basic Toxicokinetics in the Rat

Section A6.2

Annex Point IIA6.2

Table A6.2-1. Accountability of radioactivity from oral dosing of ^{14}C-benfluthrin (in vivo test)

(expressed as % of total administered dose)

Study	Sex	Urine				Faeces		Renal to faecal ratio	Tissues	Cage wash
		8 hrs	8-24 hrs	24-48 hrs	Total	24 hrs	24-48 hrs			
LDE (5 animals each gender)	M	45.4	39.7	2.9	87	8.6	2.0	8.7:1	1.6	1.2
	F	38.1	48.3	2.7	86	9.5	2.2	7.8:1	1.5	0.9
HDE (5 animals each gender)	M	41.5	44.8	4.6	87	7.7	2.8	8.7:1	1.8	1.0
	F	47.2	40.9	2.5	90	6.6	1.4	11.2:1	1.1	1.0
SHDE (2 animals each gender)	M	39.8	34.2	2.0	74	22.7	1.2	3.1:1	0.6	0.6
	F	34.9	45.9	4.4	83	13.9	1.2	5.5:1	0.8	1.1
LDCE (5 animals each gender)	M	39.5	47.4	2.1	88	8.3	2.0	8.8:1	1.3	0.6
	F	46.2	37.6	3.1	87	6.6	2.3	9.7:1	1.6	1.7
Comment:	Numbers represent the average of the group. Rounding affects the totals.									

Table A6.2-2. Metabolites from oral dosing of ^{14}C-benfluthrin (in vivo test)

(expressed as % of total administered dose)

Study	Sex	Tetrafluorobenzoic acid	Tetrafluorobenzyl glucuronide	Others	Total
LDE (5 animals each gender)	M	62	25	<1	87
	F	67	20	1	88
HDE (5 animals each gender)	M	54	30	2	86
	F	69	18	3	90
SHDE (2 animals each gender)	M	59	14	<1	73
	F	66	16	2	84
LDCE	M	50	34	4	88
	F	63	21	4	88

Comment:	Numbers represent the average of the group. Rounding affects the totals.

SECTION A 6.2/02

BPD Annex Point IIA6.2

Metabolism Study in Animal

Metabolism in female rats

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		31 REFERENCE
31.1	**Reference**	[redacted] (2009) [Methylene-14C]Transfluthrin – Metabolism in Organs and Tissues of Female Rats, [redacted], Study report No: MEF-09/483, Report date 2009-12-03 (unpublished). **BES Reference: M-359903-01-1**
31.2	**Data protection**	Yes.
31.2.1	Data owner	Bayer CropScience AG
31.2.2	Companies with letter of access	None
31.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		32 GUIDELINES AND QUALITY ASSURANCE
32.1	**Guideline study**	US EPA Health Effects Test Guideline, OPPTS 870.7485; Metabolism and Pharmacokinetics EU Council Directive 91/414/EEC amended by the Commission Directive 94/79/EC PMRA Ref.: DACO 4.5.9 Metabolism/Toxicokinetics in Mammals (Lab. Animal) OECD Guideline for Testing Chemicals No. 417, Toxicokinetics Japanese MAFF Test Guidelines for Supporting Registration of Chemical Pesticides, 12 Nousan 8147
32.2	**GLP**	Yes
32.3	**Deviations**	None.
		33 MATERIALS AND METHODS
33.1	**Test material**	
33.1.1	Lot/Batch number	Sample-ID: KATH 6316 Batch No. SEL 1520
33.1.2	Specification	As given in section 2
33.1.2.1	Description	Solid, dried in vacuo
33.1.2.2	Purity	Radiolabelled transfluthrin – radiochemical purity >99% (determined by HPLC and TLC) Non-radiolabelled transfluthrin – chemical purity >99% (determined by HPLC)
33.1.2.3	Stability	in a freezer at ≤ -18 °C until preparation of the stock solution

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33.1.2.4	Radiolabelling	Radiolabeled position: Methylene-^{14}C
33.1.2.5	Specific activity	3.67 MBq/mg = 99.19 µCi/mg = 2.2 x 10^5 dpm/µg = 36.82 Ci/mol
33.2	**Test Animals**	*Non-entry field*
33.2.1	Species	Rat (*Rattus norvegicus domesticus*)
33.2.2	Strain	Wistar Hsd/Cpb: WU
33.2.3	Source	[redacted]
33.2.4	Sex	Female
33.2.5	Age/weight at study initiation	Approx. 8 – 9 weeks (female rats) at the time of delivery 195 – 218 g at fosing
33.2.6	Number of animals per group	12
33.2.7	Control animals	No
33.3	**Administration/ Exposure**	Oral
33.3.1	Duration of treatment	Single dosing
33.3.2	Frequency of exposure	Single dosing
33.3.3	Post exposure period	1, 5 and 24 hours
33.3.4	Type	gavage
33.3.5	Concentration	3 mg/kg bw. Details are given in Table A6.2/02-1
33.3.6	Vehicle	0.5% aqueous Cremophor EL
33.3.7	Concentration in vehicle	1.54 mg/mL Specific activity: 5.67 MBq/mL (3.4 x 10^8 dpm/mL)
33.3.8	Total volume applied	2 mL
33.3.9	Controls	Not applicable
33.4	**Sample collection**	The collection intervals for the respective samples are shown in Table A6.2/02-2
33.4.1	Collection of blood	The oozed out blood obtained at sacrifice by exsanguination was collected in heparinised test tubes and immediately diluted with acetonitrile in a ratio of approx 1/1 (v/v) in order to stop any possible enzyme activity on the one hand and precipitate crude protein as well as haemolyse blood cells on the other hand. The radioactivity of the blood samples was determined after centrifugation in the supernatant directly by LSC. The radioactivity of the precipitated protein and blood cells debris fraction was measured together with the respective carcass sample by combustion/LSC
33.4.2	Collection of urine	Urine was collected at various times (0 – 1 h, 0 – 5 h, 0 – 24 h) separately for each animal in a cryogenic trap cooled with dry ice. The funnels for urine collection were rinsed with demineralised water at the end of each sampling period. The rinsing solutions were drained into the same vial as

		the corresponding urine fraction. The radioactivity was determined by LSC
33.4.3	Collection of faeces	Faeces were collected at various times (0 – 1 h, 0 – 5 h, 0 – 24 h) separately for each animal in a cryogenic trap cooled with dry ice. All individual samples were added to the respective gastrointestinal tract (GIT) of the corresponding rat at sacrifice.
33.4.4	Sample preparation at sacrifice	The dissected tissue samples (GIT including faeces, skin, and carcass (including precipitated blood protein and blood cells debris) were transferred into tared plastic vessels for straight recording of their individual fresh weights. The combined GIT/faeces-sample and an aliquot sample of depilated skin were lyophilised by freeze-drying. After weighing, they were homogenized before aliquots were taken for determination of the radioactivity by combustion/LSC. The original whole carcass/blood cells debris sample was passed up to four times through a mincing machine in half-frozen state. From this tissue pulp, an aliquot was lyophilised, homogenized and weighed, before aliquots were taken for determination of the radioactivity by combustion/LSC. Liver, kidneys and perirenal fatty tissues were weighed separately. In order to get sufficient sample material for extraction of radioactive residues and metabolic profiling, the total radioactivity values of the individual organs and tissues were not determined. Instead, pool samples of these organs and tissues were generated for each test group. The mean total dpm-values from the sum of extracts and solids of the respective samples were used for the calculations
33.5	**Sample identification, handling and storage**	All individual samples were identified with a specific sample number. Freeze-dried samples were stored in plastic vials at room temperature or at approx. +5 °C in a refrigerator. Liquid samples were kept frozen at ≤ -18 °C at all times except during aliquotation for analysis. During the analytical work the samples were stored either in a refrigerator at approx. +5 °C for a short period of time or in a freezer at approx. ≤ -18 °C.
33.6	**Measurement of radioactivity**	The measurement of the radioactivity in liquid samples was carried out by liquid scintillation counting (LSC). All solid samples were combusted in an oxygen atmosphere using an oxidiser. The released $^{14}CO_2$ was trapped in an alkaline scintillation cocktail and the radioactivity was determined by LSC.
33.7	**Quantitative evaluation**	The results of the in-life part of the study were produced by computer assistance. The validated software package **PhaLIMS** (Pharmacokinetic-LIMS) was used for planning the study, the controlled on-line data acquisition and subsequent evaluation of the data. The total amount of radioactivity dosed to the animals served as reference-value (A0 = 100%) for the percentage calculation of the total radioactivity in the biological samples. The amounts of radioactivity found in the excreta and in the organs and tissues at sacrifice were calculated from the radioactivity concentrations determined by radioassay and were related to the dosed radioactivity. The percentage amounts in the organs were obtained from the multiplication of the respective dose normalized concentrations (C_{norm}) with the corresponding gamma-values. The gamma value of an organ is equivalent to its percentage weight contribution to the total body weight of the animal. These values were determined by weighing.

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		The total radioactivity values of the individual livers, kidneys, and renal fat samples were not determined. Instead, pool samples of these organs and tissues were generated for each test group. The mean total dpm-values from the sum of extracts and solids of the respective samples were used for the calculations in PhaLIMS.
33.8	**Analytical methods**	Samples were analysed using HPLC-radiometric detection, HPLC- and GC-mass spectrometry, and ^{1}H-NMR. The HPLC methods were based on the use of a reversed phase column and an acidic acetonitrile gradient.
33.9	**Identification / Characterisation and Quantitation of Residues**	
33.9.1	Urine samples	For each test group, a combined sample was prepared from all animals. The amount of radioactivity applied for analysis was calculated from the in-life data of the respective samples. Aliquots thereof were analysed without any further sample processing by HPLC for metabolic profiling and quantitation.
33.9.2	Blood samples	For each test group, a combined sample was prepared from all animals. Every sample was concentrated with a gentle stream of nitrogen. Aliquots thereof were analysed by HPLC for metabolic profiling and quantitation.
33.9.3	Liver and Kidney samples	The livers from all animals of a test group were combined for extraction of radioactive residues. Three consecutive solvent extractions were performed by macerating the sample twice with ACN/water (8/2, v/v) and finally once with ACN using an Ultra Turrax homogenizer. At each step, the respective sample was separated into the extract and solids by centrifugation. The total volume of each extract was measured and the radioactivity of an aliquot was determined by LSC. The remaining solids 1 (PES 1) were dried, weighed and homogenized afterwards. Aliquots thereof were taken for radioactivity measurement by combustion/LSC. All extracts with values > LOQ were combined and concentrated to the aqueous remainder for HPLC analysis. The same procedure was applied to kidneys
33.9.4	Fat samples	The perirenal fatty tissues from all animals of a test group were combined for extraction of radioactive residues. Three consecutive solvent extractions were performed by macerating the sample twice with ACN/water (8/2, v/v) and finally once with ACN using an Ultra Turrax homogenizer. At each step, the respective sample was separated into the extract and solids by filtration. The total volume of each extract was measured and the radioactivity of an aliquot was determined by LSC. The remaining solids 1 (PES 1) were dried, weighed and homogenized afterwards. Aliquots thereof were taken for radioactivity measurement by combustion/LSC. All extracts with values > LOQ were combined and applied to a C18 SPE cartridge to remove the lipid fraction of the matrix. The SPE percolate and column ACN/water rinse were combined and concentrated to the aqueous remainder for HPLC analysis.

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34 RESULTS AND DISCUSSION

34.1 Dosing level

The actual dose level was near to the calculated dose of 3.0 mg/kg bw. Each rat in the experiments received on average an actual amount of 0.61 mg [methylene-^{14}C]Transfluthrin, corresponding to a dose of 2.93 mg/kg bw, respectively. See Table A6.2/02-1:

34.2 Recovery

The entire balances of all tests are shown in Table A6.2/02-3: Between 93.87% (test 1) and 101.30% (test 3) of the administered dose were recovered from measurement of the total radioactivity in urine and blood as well as in organs and tissues at sacrifice.

34.3 Time course of total Radioactivity

The total radioactivity was determined in the urines of different time periods likewise. During the experimental period of 24 hours, a steady increase up to 87.66% of the dose was measured (Table A6.2/02-3 and Figure A6.2/02-1) which confirmed that the urinary excretion is the preferred path for the excretion.

As a measure for systemic exposure to Transfluthrin and its possible metabolites, the total radioactivity was determined in blood. Values were calculated for the percentage of administered dose (Table A6.2/02-3), equivalent concentration (C) and dose normalized concentration (C_{norm}). A summary for C and C_{norm} is shown in Table A6.2/02-4 and Figure A6.2/02-2).

The highest levels for all three categories were found at 1 hour after dosing that decreased until 24 hours by several orders of magnitude (a factor of approx. 50 for C_{norm}).

34.4 Radioactivity in tissue samples

The total radioactive residues (expressed as percentage of administered dose, equivalent concentration C (= TRR) and dose normalized concentration C_{norm}) detected in the organs and tissues at sacrifice are given in Table A6.2/02-3 and Table A6.2/02-4. A diagram referring to the TRR-values of radioactive residues in organs and tissues is shown in Figure A6.2/02-3.

At 1 hour after dosing, approx. 25.7% and 48.4% of the dose were detected in the organs and tissues as well as in the combined GIT plus faeces sample, respectively. Until 24 hours, a significant decrease in both samples to approx. 1.15% and 12.5% was measured.

The highest TRR-values were detected in the organs and tissues at the initial time-point with the exception of perirenal fat that peaked at 5 hours after dosing. All values decreased significantly over 24 hours (See Table A6.2/02-5).

34.5 Extraction efficiency and identification strategy

Combined urine and blood samples of the individual tests were analysed directly by HPLC.

Following conventional extraction of liver, kidney and perirenal fat, the resulting extracts represented between approx. 30% and 99% of the TRR. For the liver samples, the extractability by this standard method decreased from approx. 96% (1 h) to 71% (24 hrs). In case of kidney samples , the extraction efficiency values decreased from approx. 96% (1 h) to 30% (24 hrs) and of perirenal fat samples from approx. 99% (1 h) to 74% (24 hrs). The extracts were concentrated and analysed afterwards by HPLC.

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The following strategy was used for identification of parent compound and metabolites. The assignment of the unchanged parent compound and metabolites in the samples was achieved by

- a) direct LC-MS of the 1 hour urine sample , the 5 hours kidney extract and the 5 hours perirenal fat extract ,
- b) HPLC co-chromatography of selected samples with non-radiolabelled and radiolabelled reference compounds. The radiolabelled reference compounds were isolated from the 1 hour blood sample (test 1) and the 1 hour liver extract by semipreparative HPLC and identified by LC-MS.
- c) comparison of the HPLC profiles

Further small peaks or peak groups additionally detected in the HPLC profiles were characterised by their behaviour during extraction and clean up and the retention times in the HPLC chromatograms

34.6 Profiles and quantitation of metabolites

All identified and unknown metabolites were quantitatively determined in composite samples of urine, blood, and extracts from liver, kidney and perirenal fat. For quantitation, the ^{14}C-signals in the HPLC chromatograms were integrated. Corresponding compounds in the samples were labelled with the same peak number.

34.6.1 Metabolites in Urine

The unchanged parent compound was not detected in any sample. The cleavage components Transfluthrin-tetrafluorobenzyl-glucuronide and Transfluthrin-tetrafluorobenzoic acid were the major metabolites in all samples. The metabolite Transfluthrin-tetrafluorobenzoic acid was by a factor of 2 (test 2) to 4.6 (test 3) higher than Transfluthrin-tetrafluorobenzyl-glucuronide. Very minor amounts of the metabolites acetyl-carboxylic acid (isomer 2) and hydroxymethyl-glucuronide were additionally verified. See Table A6.2/02-6

34.6.2 Metabolites in Blood

The unchanged parent compound was not detected in any sample. The major components of the 1-hour sample with more than 0.1 mg/kg were identified as Transfluthrin-tetrafluorobenzoic acid (TFBA) (0.525 mg/kg) and both isomers of Transfluthrin-carboxylic acid. Lower values were calculated for all other metabolites. The metabolites from the 1-hour sample were also found in the 5-hours sample, however in significant lower concentrations. At 24 hours, the two isomers of Transfluthrin-carboxylic acid were the only detectable metabolites. The sum of all metabolites with an uncleaved parent compound structure increased from approx. 32% of the TRR at 1 hour to 57% at 5 hours and 95% at 24 hours. A minor amount of the metabolites acetyl-carboxylic acid (isomer 2) and hydroxymethyl-glucuronide was additionally verified. See Table A6.2/02-7

34.6.3 Metabolites in Liver

The unchanged parent compound was not detected in any sample. The major components of the 1-hour liver extract with more than 0.3 mg/kg were identified as Transfluthrin-tetrafluorobenzylalcohol (0.909 mg/kg), the isomer 1 of Transfluthrin-carboxylic acid (0.448 mg/kg) and Transfluthrin-tetrafluorobenzoic acid (0.340 mg/kg). Lower values were calculated for all other metabolites. The metabolites from the 1-hour sample were also found in the 5-hours sample, most of them in significantly lower concentrations. One exception was the isomer 1 of Transfluthrin-carboxylic acid for which the amount increased to 0.497 mg/kg. This metabolite was also the only component with more than 0.1 mg/kg in the 24 hours sample. The sum of all metabolites with an uncleaved parent compound structure increased

		from approx. 35% of the TRR at 1 hour to 58% at 5 hours and 67% at 24 hours. See Table A6.2/02-8
34.6.4	Metabolites in Kidney	The unchanged parent compound was not detected in any sample. The major components of the 1-hour kidney extract with more than 0.1 mg/kg were identified as Transfluthrin-tetrafluorobenzoic acid (1.946 mg/kg), Transfluthrin-tetrafluorobenzyl-glucoronide (0.729 mg/kg) and Transfluthrin-tetrafluorobenzylalcohol (0.116 mg/kg). The residue levels for all other metabolites were lower than 0.1 mg/kg. The metabolites from the 1-hour sample were also found in the 5-hours sample in which the three benyzl ring metabolites mentioned before were again the prominent components. Transfluthrin-tetrafluorobenzoic acid and the isomer 1 of Transfluthrin-carboxylic acid were the only metabolites detected in the 24-hours sample. The sum of all metabolites with an uncleaved parent compound structure increased from approx. 5.4% of the TRR at 1 hour to 9.7% at 5 hours and 10.7% at 24 hours. See Table A6.2/02-9
34.6.5	Metabolites in Perirenal fat	The unchanged parent compound was detected in all samples with the highest amount in the 1-hour sample (0.044 mg/kg). In this sample, the major metabolite was Transfluthrin-tetrafluorobenzylalcohol (0.237 mg) which was followed by Transfluthrin-tetrafluorobenzoic acid (0.066 mg/kg). Significant lower values were calculated for both isomers of Transfluthrin-carboxylic acid. The three benzyl ring metabolites mentioned above were the only components in the 5- and 24-hours samples. From these Transfluthrin-tetrafluorobenzoic acid and Transfluthrin-tetrafluorobenzyl-glucuronide showning the highest amounts. See Table A6.2/02-10
34.7	**Metabolic pathway**	The principal metabolic reactions of [methylene-^{14}C]Transfluthrin in the female rat were: ▪ ester cleavage of the molecule to form Transfluthrin-tetrafluorobenzylalcohol ▪ conjugation of Transfluthrin-tetrafluorobenzylalcohol with glucuronic acid ▪ further oxidation Transfluthrin-tetrafluorobenzylalcohol to Transfluthrin-tetrafluorobenzoic acid ▪ hydroxylation of a methyl group of the cyclopropane ring followed by glucuronidation to the hydroxymethyl-glucuronide ▪ oxidation of the hydroxymethyl group of cyclopropane ring to the carboxylic acid ▪ oxidative and reductive dehalogenation of the dichlorovinyl side chain The positions in the molecule, which are involved in the metabolic reactions, are schematicall described in Figure A6.2/02-4. The proposed metabolic pathway of [methylene-^{14}C]Transfluthrin is presented in Figure A6.2/02-5.
		35 APPLICANT'S SUMMARY AND CONCLUSION
35.1	**Materials and methods**	the toxicokinetic behaviour and metabolism of transfluthrin were investigated in female Wistar rats. The test compound was labelled with ^{14}C in the methylene C-atom of the molecule as shown below:

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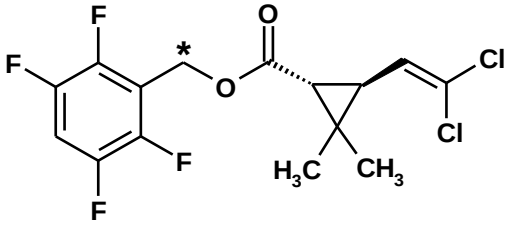

✱ denotes the ^{14}C-label position

The test compound was orally dosed as an emulsion (0.5% aqueous Cremophor EL®) at a target dose level of 3 mg/kg body weight. The purpose of the study was to examine the amount and nature of radioactive residues in blood, liver, kidney and perirenal fat for selected time-points between dosing and sacrifice. Additionally, the total radioactive residues were determined in the carcass (plus blood cells debris), gastrointestinal tract (plus faeces of the respective collection period) and skin at sacrifice. For comparison reasons with the former ADME rat studies, the urinary excretion including their metabolic profiles was investigated.

The study was performed according to the current EPA, EU and OECD test guidelines for supporting the registration of chemical pesticides. A total of twelve animals (4 rats/test) were used. The animals were sacrificed at the latest after 24 hours. Following samples were collected at various intervals: urine (0 – 1 h, 0 – 5 h, 0 – 24 h); faeces (0 – 1 h, 0 – 5 h, 0 – 24 h); blood, liver, kidney, perirenal fat, skin, carcass, GIT: all at sacrifice

The collection intervals for the respective samples are shown in Table A6.2/02-2

35.2 Results and discussion

The overall recovery accounted for approx. 93.9% to 101.3% of the administered dose. The entire balances for the total radioactivity detected in urines, the combined GIT plus faeces samples, skins, and organs and tissues are shown in Table A6.2/02-3.

Time course of total radioactivity in urine

The total radioactivity was determined in urines of different collection periods. During the experimental period of 24 hours, a steady increase up to approx. 87.7% of the administered dose was measured. This confirmed similar results obtained in former Transfluthrin ADME rat studies [14 and 15]

[14] Ecker W., Prinz H., Bornatsch W. (1997) [Methylene-^{14}C]Transfluthrin: Biokinetic behaviour and metabolism in the rat after i.v. administration, Bayer PF-report no.: 4257 / BES Ref. MO-03-009851 (non-key study summarised in OUCLID file)

[15] Minor R. G., Freeseman P. L. (1991) Disposition of [methylene-14C]Benfluthrin (NAK 4455) in rats, Mobay Corp.-report no.: 101310, BES Ref: MO-03-010378 (key study, see A6.2-01)

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Time course of total radioactivity in blood

As a measure for systemic exposure to Transfluthrin and its possible metabolites, the total radioactivity was determined in blood. Blood and not plasma was chosen as matrix, since Transfluthrin is most likely cleaved in blood or plasma by esterases. In order to avoid ongoing enzymatic cleavage during sample preparation each blood sample was mixed immediately after collection in a ratio of 1:1 (v/v) with acetonitrile thus leading to protein precipitation, haemolysis of blood cells and stoppage of the cleavage reaction. Haemolysed blood cells (debris) and precipitated enzymes were removed by centrifugation and measured together with the respective carcass sample. All resulting soluble radioactivity in blood referring to Transfluthrin and its metabolites was determined in the supernatant fraction taking into account a dilution factor of 2 for calculation of the equivalent concentrations (C) and dose normalized concentrations (C_{norm}). A summary is shown in the following table:

		Blood (mean values)		
Test no.	Time [h post admin.]	% of dose administered	Equiv. conc. C [mg/kg$_{sample}$]	C_{norm}
1	1	0.65	1.034	0.358
2	5	0.16	0.248	0.084
3	24	0.02	0.025	0.008

The highest levels for all three categories were found at 1 hour after dosing. They decreased significantly until 24 hours by a factor of approximately 50 for C_{norm}. The toxicokinetic behaviour of the parent compound related radioactivity can therefore be characterised by a fast uptake and distribution followed by quick elimination.

Total radioactive residues in organs and tissues

At 1 hour after dosing, approx. 25.7% and 48.4% of the dose were detected in the organs and tissues as well as in the combined GIT plus faeces sample, respectively. Until 24 hours, a significant decrease in both samples to approx. 1.2% and 12.5% was measured.

The highest TRR-values were detected in the organs and tissues at the initial time-point with the exception of perirenal fat that peaked at 5 hours after dosing. As shown in Table A6.2/02-3., all values decreased significantly until 24 hours. No indications of irreversible binding or retention of radioactivity in organs and tissues of the rat were recognisable. It is therefore expected that the residual amounts will be further eliminated smoothly from the body.

Metabolism

Samples were analysed using HPLC-radiometric detection, HPLC- and GC-mass spectrometry, and 1H-NMR. The HPLC methods were based on the use of a reversed phase column and an acidic acetonitrile gradient.

Combined urine and blood samples of the individual tests were analysed

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directly by HPLC. Composite samples of liver, kidney and perirenal fat were successively extracted twice with acetonitrile/water (80/20; v/v) and finally once with acetonitrile. The resulting extracts represented between approx. 30% and 99% of the TRR. For the liver samples, the extractability by this standard method decreased from approx. 96% (1 h) to 71% (24 h), for the kidney from approx. 96% (1 h) to 30% (24 h) and for the perirenal fat from approx. 99% to 74%. The extracts were concentrated and analysed by HPLC with radiodetection.

Parent compound and metabolites were identified by HPLC-mass spectrometry, co-chromatography with authentic non-radiolabelled reference compounds, co-chromatography with radiolabelled metabolites isolated from blood samples and liver extracts of this study, and by comparison of the HPLC-profiles among each other.

A small amount of unchanged parent compound was detected only in the perirenal fatty tissue in which it was obviously protected from further metabolism.

All major and several minor metabolites were identified. Identification rates ranged to 100% of the TRR in urine and blood, from approx. 71% to 91% in liver, approx. 23% to 92% in kidney and approx. 74% to 99% in perirenal fat.

35.3 Conclusion

The principal metabolic reactions of [methylene-^{14}C]Transfluthrin in the female rat were:

- ester cleavage of the molecule to form Transfluthrin-tetrafluorobenzylalcohol
- conjugation of Transfluthrin-tetrafluorobenzylalcohol with glucuronic acid
- further oxidation Transfluthrin-tetrafluorobenzylalcohol to Transfluthrin-tetrafluorobenzoic acid
- hydroxylation of a methyl group of the cyclopropane ring followed by glucuronidation to the hydroxymethyl-glucuronide
- oxidation of the hydroxymethyl group of cyclopropane ring to the carboxylic acid
- oxidative and reductive dehalogenation of the dichlorovinyl side chain

The detection of Transfluthrin in fat and of significant proportions of metabolites with the uncleaved ester moiety demonstrate that unchanged Transfluthrin is the major part of radioactivity absorbed from the gastrointestinal tract after oral dosing of the test compound.

With regard to urine, transfluthrin-tetrafluorobenzyl-glucuronide and transfluthrin-tetrafluorobenzoic acid were identified as the only metabolites as well.

A metabolic pathway is proposed and shown in Figure A6.2/02-5.

35.3.1 Reliability: 1

35.3.2 Deficiencies: No

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2010
Materials and Methods	The version of the applicant is acceptable
Results and discussion	
Conclusion	RMS supports the conclusion.
Reliability	
Acceptability	Acceptable
Remarks	See doc IIIA 6.10 appendices with position papers mechanistic considerations dated 19-02-2010 and 10-05-2010. Furthermore the appendix interpretation of short-term assays regarding the effects of transfluthrin on rat urethelium in vivo and in vitro by [redacted] March 12, 2010. RMS supports the conclusions described in the position papers.
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.2/02-1: Dosing level

Test no.	Dosing emulsion							
	Total prepared		Dosed per rat					
	Amount [mg]	Volume [mL]	Volume/ Amount	Target dose mg/kg bw	Radioactivity [dpm]	Amount [mg]	Actual dose [mg/kg bw]	Mean rat weight [g]
1	10.8	18	1.0 mL/0.6 mg	3	133 495 112	0.61	2.86	212
2							2.89	210
3							3.06	198
Average tests 1 – 3							**2.93**	**207**

Table A6.2/02-2: Sample collection intervals

Test no.	Date of dosing	Time of sacrifice [h p. admin.]	Animal no's.	Samples collected	Collection period urine and faeces [h p. admin.]
1	2009-06-30	1	835 -838	Blood and urine; liver, kidney, perirenal fat, skin, carcass plus blood cells debris, GIT plus faeces	0 – 1 h
2	2009-06-30	5	839 - 842	same as in test 1	0 – 5 h
3	2009-06-30	24	843 - 846	same as in test 1	0 – 24 h

Table A6.2/02-3: Tests 1 – 3: Balance of radioactivity in urine, blood, and in organs and tissues of female rats sacrificed 1 h, 5 h, and 24 h after a single oral dosing

Test number	**1**	**2**	**3**
No. of animals	**4**	**4**	**4**
Sacrifice [h post dosing]	**1**	**5**	**24**
Sampling period urine [h]	**0 - 1**	**0 - 5**	**0 - 24**
Percent of radioactive dose administered (mean values)			
Excretion			
Urine	19.78	61.74	87.66
Samples			
Blood	0.65	0.16	0.02
Carcass + blood cells debris	17.33	6.90	0.54
Kidneys	0.84	0.25	0.05
Liver	3.68	1.75	0.40
Perirenal fat	0.07	0.07	0.01
GIT + faeces	48.37	22.80	12.46
Skin	3.15	0.88	0.13
Balance	**93.87**	**94.54**	**101.30**
Body excluding GIT + faeces	25.72	9.99	1.15

Table A6.2/02-4: Tests 1 – 3: Total radioactive residues blood, and in organs and tissues of female rats sacrificed 1 h, 5 h, and 24 h after a single oral dosing

Test number	1	2	3
No. of animals	4	4	4
Sacrifice [h post dosing]	1	5	24
Test number	1	2	3
No. of animals	4	4	4
Sacrifice [h post dosing]	1	5	24
Equivalent concentration [mg a.s. equiv. /kg$_{sample}$]			
Samples			
Blood	1.034	0.248	0.025
Carcass + blood cell debris	0.803	0.324	0.027
Kidneys	3.212	0.985	0.218
Liver	2.647	1.345	0.267
Perirenal fat	0.377	0.399	0.049
Skin	0.408	0.115	0.018
Dose normalized concentration			
Samples			
Blood	0.358	0.084	0.008
Carcass + blood cell debris	0.279	0.111	0.009
Kidneys	1.114	0.336	0.073
Liver	0.918	0.458	0.090
Perirenal fat	0.131	0.136	0.016
Skin	0.142	0.039	0.006

Table A6.2/02-5: TRR values at 1h and 24h after dosing

Test number Sacrifice [h post admin.]	1 1	3 24	Ratio 1/24 h	Decline by [%]
Samples	TRR [mg a.s. equiv. /kg$_{sample}$]			
Blood	1.034	0.025	41	98
Carcass plus blood cells debris	0.803	0.027	30	97
Kidneys	3.212	0.218	15	93
Liver	2.647	0.267	10	90
Perirenal fat	0.377	0.049	8	87
Skin	0.408	0.018	22	95

Table A6.2/02-6: Tests 1 – 3: Radioactive residues of parent compound and metabolites in urine

Test no.		1		2		3	
Sacrifice [h p. dosing]		1 h		5 h		24 h	
Sampling period [h]		0 - 1 h		0 - 5 h		0 - 24 h	
Total radioactive residue in urine pool sample		% of dose	% of TRR	% of dose	% of TRR	% of dose	% of TRR
		19.78	**100.00**	**61.74**	**100.00**	**87.66**	**100.00**
Peak ID	**Report name** (Transfluthrin-)						
6	tetrafluorobenzyl-glucuronide	3.75	18.96	20.25	32.79	15.77	17.99
7	tetrafluorobenzoic acid	15.92	80.49	40.94	66.30	71.89	82.01
17	acetyl-carboxylic acid (isomer 2) + hydroxy-methyl-glucuronide	0.11	0.55	0.56	0.90	---	---
22	Transfluthrin	---	---	---	---	---	---
Total		19.78	100.00	61.74	100.00	87.66	100.00
Sum identified		**19.78**	**100.00**	**61.74**	**100.00**	**87.66**	**100.00**
Sum metabolites of benzyl ring after molecular cleavage		19.67	99.45	61.18	99.10	87.66	100.00
Sum metabolites with uncleaved test compound structure		0.11	0.55	0.56	0.90	---	---

Table A6.2/02-7: Tests 1 – 3: Radioactive residues of parent compound and metabolites in blood

Test no.		1			2			3		
Sacrifice [h p. dosing]		1 h			5 h			24 h		
Total radioactive residue in blood pool sample		% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg
		0.647	**100.00**	**1.034**	**0.157**	**100.00**	**0.248**	**0.016**	**100.00**	**0.025**
Peak ID	**Report name** (Transfluthrin-)									
6	tetrafluorobenzyl-glucuronide	0.026	4.00	0.041	0.005	3.43	0.009	---	---	---
7	tetrafluorobenzoic acid	0.329	50.81	0.525	0.046	29.08	0.072	---	---	---
9	tetrafluorobenzylalcohol	0.053	8.18	0.085	0.008	5.41	0.013	---	---	---
18	acetyl-carboxylic acid (isomer 2) + hydroxy-methyl-glucuronide	0.026	4.02	0.042	0.009	5.61	0.014	0.001	5.368	0.001
19	chloroethyl-carboxylic acid	0.018	2.72	0.028	0.006	3.83	0.010	---	---	---
20	carboxylic acid (isomer 1)	0.076	11.75	0.121	0.052	33.27	0.083	0.014	85.76	0.022
21	carboxylic acid (isomer 2)	0.113	17.40	0.180	0.030	19.37	0.048	0.001	8.87	0.002
22	Transfluthrin	---	---	---	---	---	---	---	---	---

Total	0.647	100.00	1.034	0.157	100.00	0.248	0.016	100.00	0.025
Sum identified	**0.640**	**98.87**	**1.022**	**0.157**	**100.00**	**0.248**	**0.016**	**100.00**	**0.025**
Sum characterised *)	0.007	1.13	0.012	---	---	---	---	---	---
Sum metabolites of benzyl ring after molecular cleavage	0.408	62.99	0.651	0.059	37.92	0.094	---	---	---
Sum metabolites with uncleaved test compound structure	0.232	35.89	0.371	0.097	62.08	0.154	0.016	100.00	0.025

*) Peak (1) was characterized based on his retention time in HPLC-analysis:
it accounted for ≤ 1.13% of the TRR.

Table A6.2/02-8: Tests 1 – 3: Radioactive residues of parent compound and metabolites in liver

Test no.		1			2			3		
Sacrifice [h p. dosing]		1 h			5 h			24 h		
Total radioactive residue in liver pool sample		% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg
		3.682	**100.00**	**2.647**	**1.745**	**100.00**	**1.345**	**0.399**	**100.00**	**0.267**
Peak ID	**Report name** (Transfluthrin-)									
6	tetrafluorobenzyl-glucuronide	0.262	7.12	0.189	0.072	4.13	0.055	---	---	---
7	tetrafluorobenzoic acid	0.473	12.85	0.340	0.106	6.09	0.082	---	---	---
9	tetrafluorobenzylalcohol	1.265	34.35	0.909	0.406	23.29	0.313	0.014	3.62	0.010
16	hydroxyethyl-carboxylic acid + acetyl-carboxylic acid (isomer 1)	0.148	4.02	0.106	0.093	5.31	0.071	0.006	1.41	0.004
17	acetyl-carboxylic acid (isomer 2) + hydroxymethyl-glucuronide	0.107	2.90	0.077	0.055	3.18	0.043	---	---	---
18		0.243	6.59	0.174	0.119	6.85	0.092	0.009	2.19	0.006
19	chloroethyl-carboxylic acid	0.106	2.89	0.076	0.064	3.67	0.049	0.003	0.82	0.002
20	carboxylic acid (isomer 1)	0.623	16.92	0.448	0.645	36.99	0.497	0.251	62.85	0.168
21	carboxylic acid (isomer 2)	0.062	1.67	0.044	0.029	1.65	0.022	---	---	---
22	Transfluthrin	---	---	---	---	---	---	---	---	---
Total		3.529	95.84	2.537	1.661	95.21	1.281	0.283	70.89	0.190
Sum identified		**3.289**	**89.31**	**2.364**	**1.591**	**91.16**	**1.226**	**0.283**	**70.89**	**0.190**
Sum characterised *)		0.240	6.52	0.173	0.071	4.05	0.054	---	---	---
Solids 1 (PES 1)		0.153	4.16	0.110	0.084	4.79	0.064	0.116	29.11	0.078
Sum total		**3.682**	**100.00**	**2.647**	**1.745**	**100.00**	**1.345**	**0.399**	**100.00**	**0.267**
Sum metabolites of benzyl ring after molecular cleavage		2.000	54.33	1.438	0.585	33.51	0.451	0.014	3.62	0.010
Sum metabolites with uncleaved test compound structure		1.288	34.99	0.926	1.006	57.65	0.775	0.268	67.27	0.180

*) Peaks (10) were characterized based on their retention time in HPLC-analysis: none of them accounted for ≥ 1.52% of the TRR.

Table A6.2/02-9: Tests 1 – 3: Radioactive residues of parent compound and metabolites in kidney

Test no.		1			2			3		
Sacrifice [h p. dosing]		1 h			5 h			24 h		
Total radioactive residue in kidney pool sample		% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg
		0.838	**100.00**	**3.212**	**0.247**	**100.00**	**0.985**	**0.053**	**100.00**	**0.218**
Peak ID	**Report name** (Transfluthrin-)									
6	tetrafluorobenzyl-glucuronide	0.190	22.71	0.729	0.044	17.83	0.176	---	---	---
7	tetrafluorobenzoic acid	0.508	60.59	1.946	0.103	41.57	0.410	0.006	11.91	0.026
9	tetrafluorobenzylalcohol	0.030	3.62	0.116	0.029	11.84	0.117	---	---	---
16	hydroxyethyl-carboxylic acid + acetyl carboxylic acid (isomer 1)	0.004	0.42	0.013	0.003	1.17	0.012	---	---	---
17	acetyl-carboxylic acid (isomer 2) + hydroxymethyl-glucuronide	0.003	0.37	0.012	---	---	---	---	---	---
18		0.009	1.05	0.034	0.004	1.78	0.018	---	---	---
19	chloroethyl-carboxylic acid	0.010	1.22	0.039	0.003	1.41	0.014	---	---	---
20	carboxylic acid (isomer 1)	0.016	1.92	0.062	0.012	4.95	0.049	0.006	10.70	0.023
21	carboxylic acid (isomer 2)	0.004	0.47	0.015	0.001	0.36	0.004	---	---	---
22	Transfluthrin	---	---	---	---	---	---	---	---	---
Total		0.807	96.30	3.093	0.209	84.57	0.833	0.016	30.07	0.065
Sum identified		**0.774**	**92.35**	**2.966**	**0.200**	**80.92**	**0.797**	**0.012**	**22.61**	**0.049**
Sum characterised *)		0.033	3.95	0.127	0.009	3.66	0.036	0.004	7.46	0.016
Solids 1 (PES 1)		0.031	3.70	0.119	0.038	15.43	0.152	0.037	69.93	0.152
Sum total		**0.838**	**100.00**	**3.212**	**0.247**	**100.00**	**0.985**	**0.053**	**100.00**	**0.218**
Sum metabolites of benzyl ring after molecular cleavage		0.728	86.91	2.792	0.176	71.24	0.702	0.006	11.91	0.026
Sum metabolites with uncleaved test compound structure		0.046	5.44	0.175	0.024	9.68	0.095	0.006	10.70	0.023

*) Peaks (6) were characterized based on their retention time in HPLC-analysis: none of them accounted for ≥ 1.62% of the TRR.

Table A6.2/02-10: Tests 1 – 3: Radioactive residues of parent compound and metabolites in perirenal fat

Test no.		1			2			3		
Sacrifice [h p. dosing]		1 h			5 h			24 h		
Total radioactive residue in perirenal fat pool sample		% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg	% of dose	% of TRR	mg/kg
		0.070	**100.00**	**0.377**	**0.071**	**100.00**	**0.399**	**0.007**	**100.00**	**0.049**
Peak ID	**Report name** (Transfluthrin-)									
6	tetrafluorobenzyl-glucuronide	---	---	---	0.025	34.45	0.138	0.002	24.50	0.012
7	tetrafluorobenzoic acid	0.012	17.62	0.066	0.040	55.80	0.223	0.003	40.56	0.020
9	tetrafluorobenzylalcohol	0.044	63.01	0.237	0.004	6.01	0.024	<0.001	5.36	0.003
20	carboxylic acid (isomer 1)	0.001	1.75	0.007	---	---	---	---	---	---
21	carboxylic acid (isomer 2)	0.003	4.759	0.018	---	---	---	---	---	---
22	Transfluthrin	0.008	11.62	0.044	0.002	3.05	0.012	<0.001	3.25	0.002
Total		0.069	98.77	0.372	0.071	99.31	0.397	0.005	73.67	0.036
Sum identified		**0.069**	**98.77**	**0.372**	**0.071**	**99.31**	**0.397**	**0.005**	**73.67**	**0.036**
Solids 1 (PES 1)		0.001	1.23	0.005	0.000	0.69	0.003	0.002	26.33	0.013
Sum total		**0.070**	**100.00**	**0.377**	**0.071**	**100.00**	**0.399**	**0.007**	**100.00**	**0.049**
Sum metabolites of benzyl ring after molecular cleavage		0.056	80.63	0.304	0.069	96.26	0.384	0.005	70.43	0.034
Sum parent and metabolites with uncleaved test compound structure		0.013	18.13	0.068	0.002	3.05	0.012	<0.001	3.25	0.002

Figure A6.2/02-1: Tests 1 – 3: Time course of total radioactivity in urine

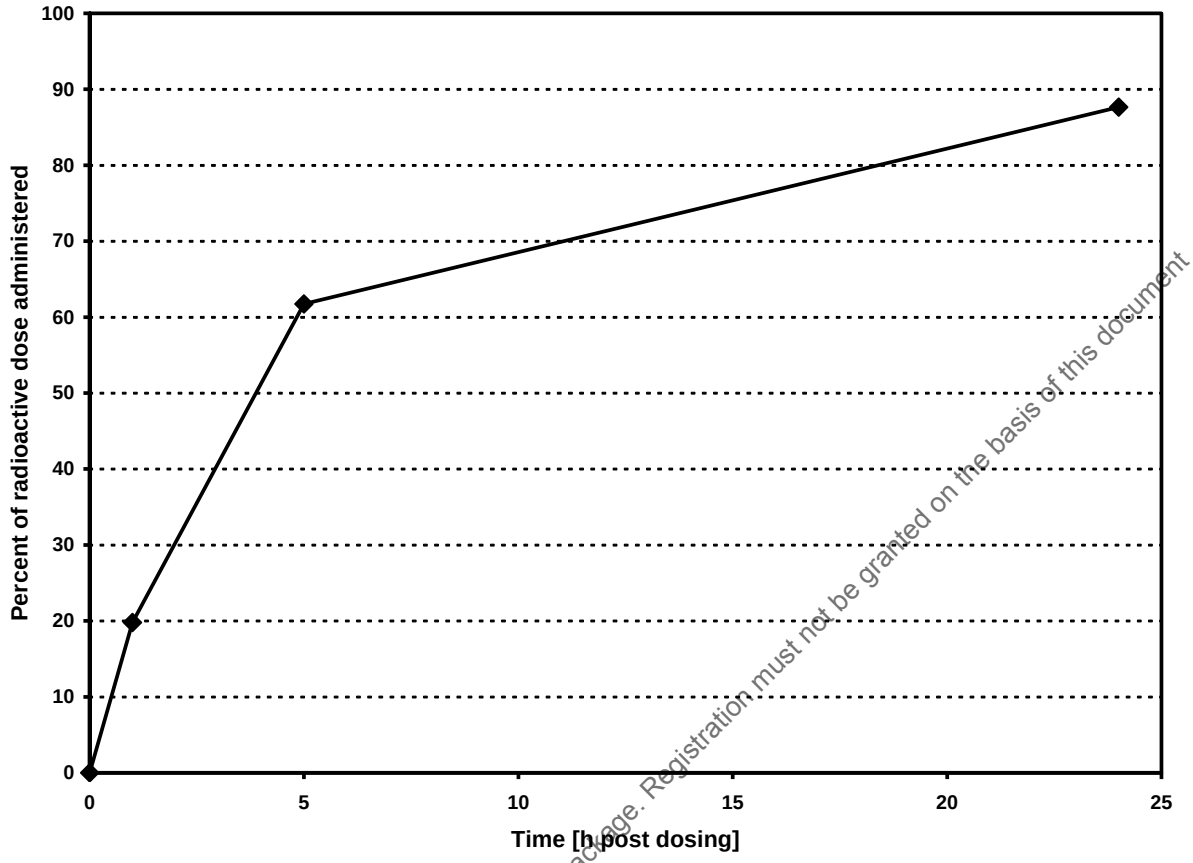


Figure A6.2/02-2: Tests 1 – 3: Time course of total radioactivity in blood

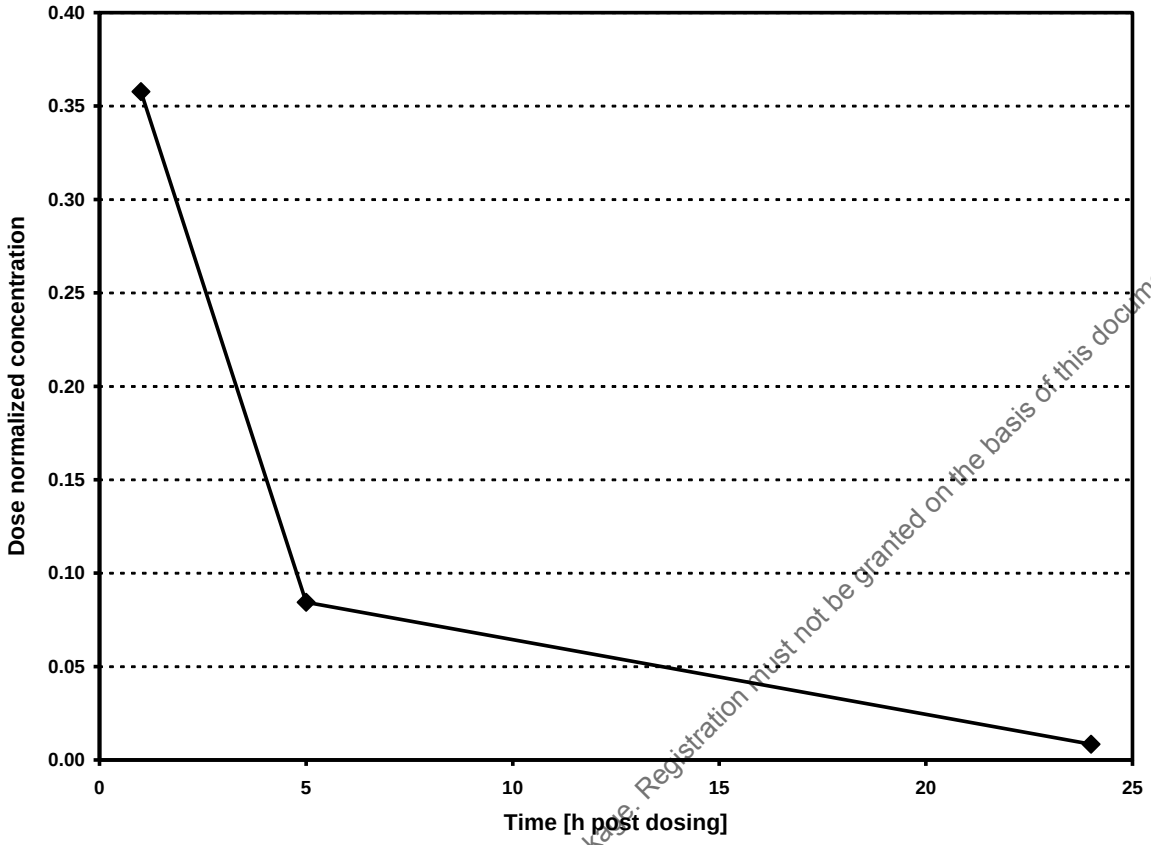


Figure A6.2/02-3: Tests 1 – 3: Total radioactive residues in blood, and in organs and tissues at sacrifice

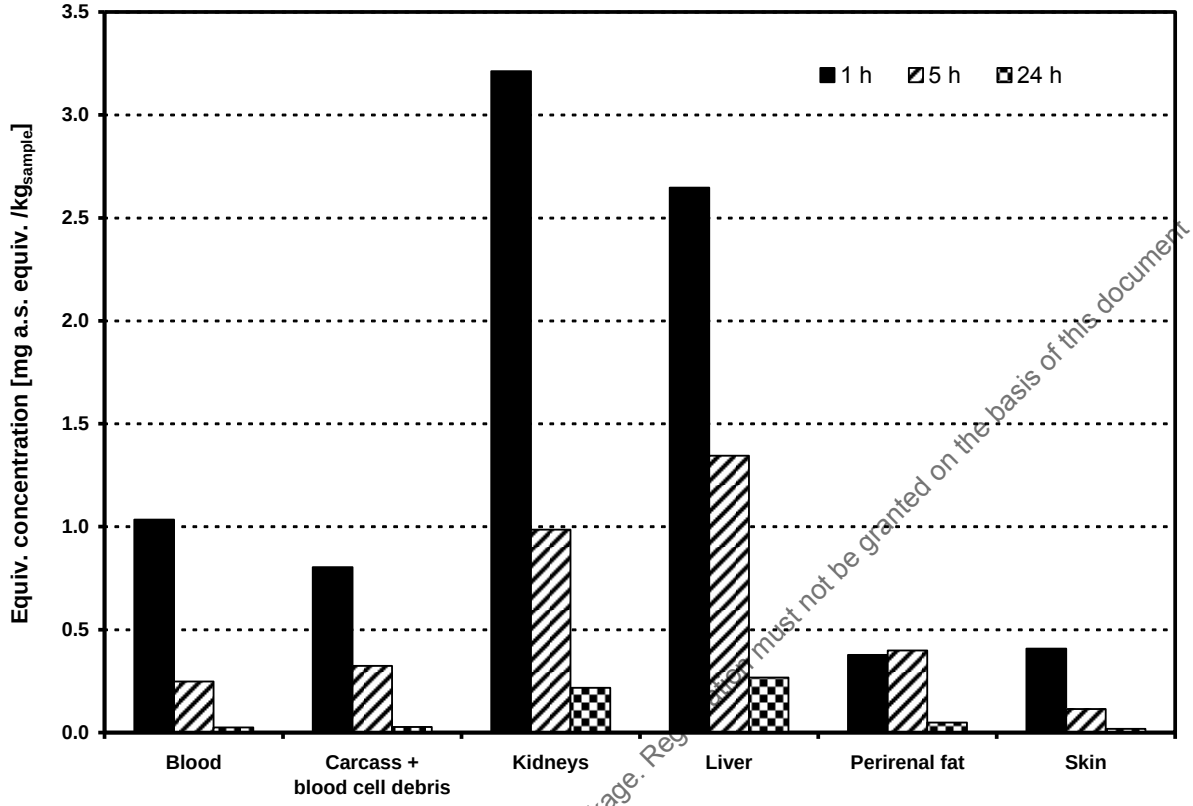


Figure A6.2/02-4: positions in the molecule, which are involved in the metabolic reactions

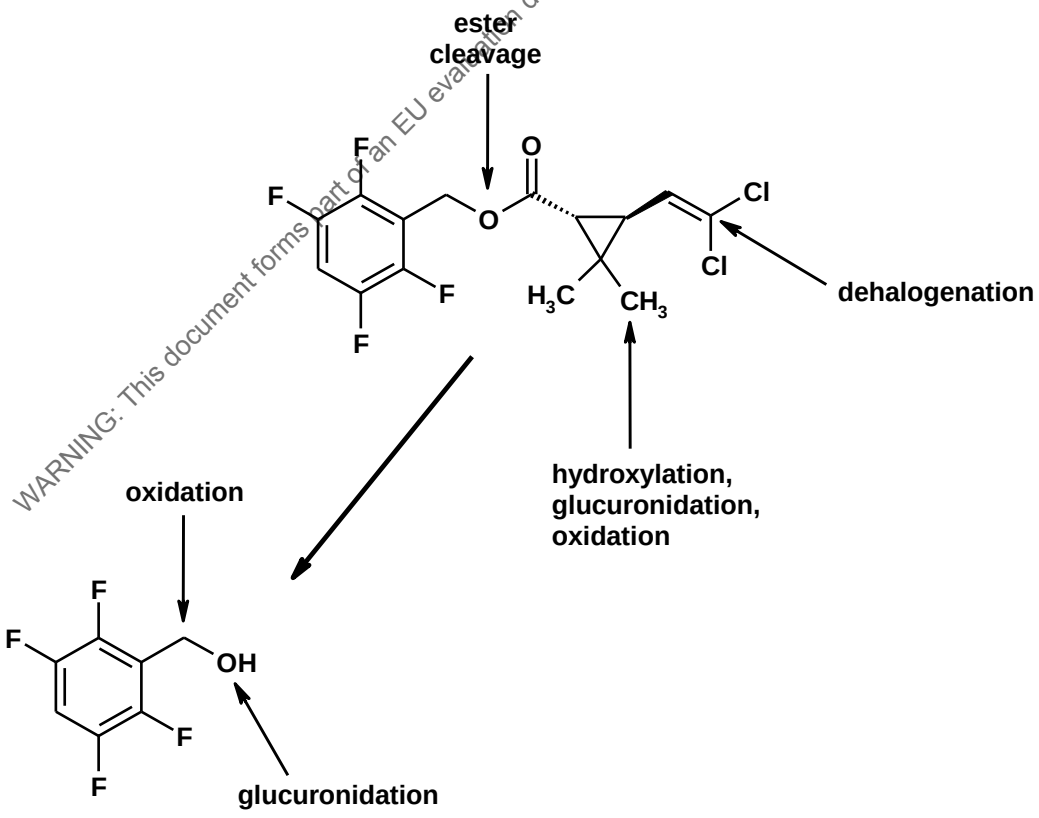
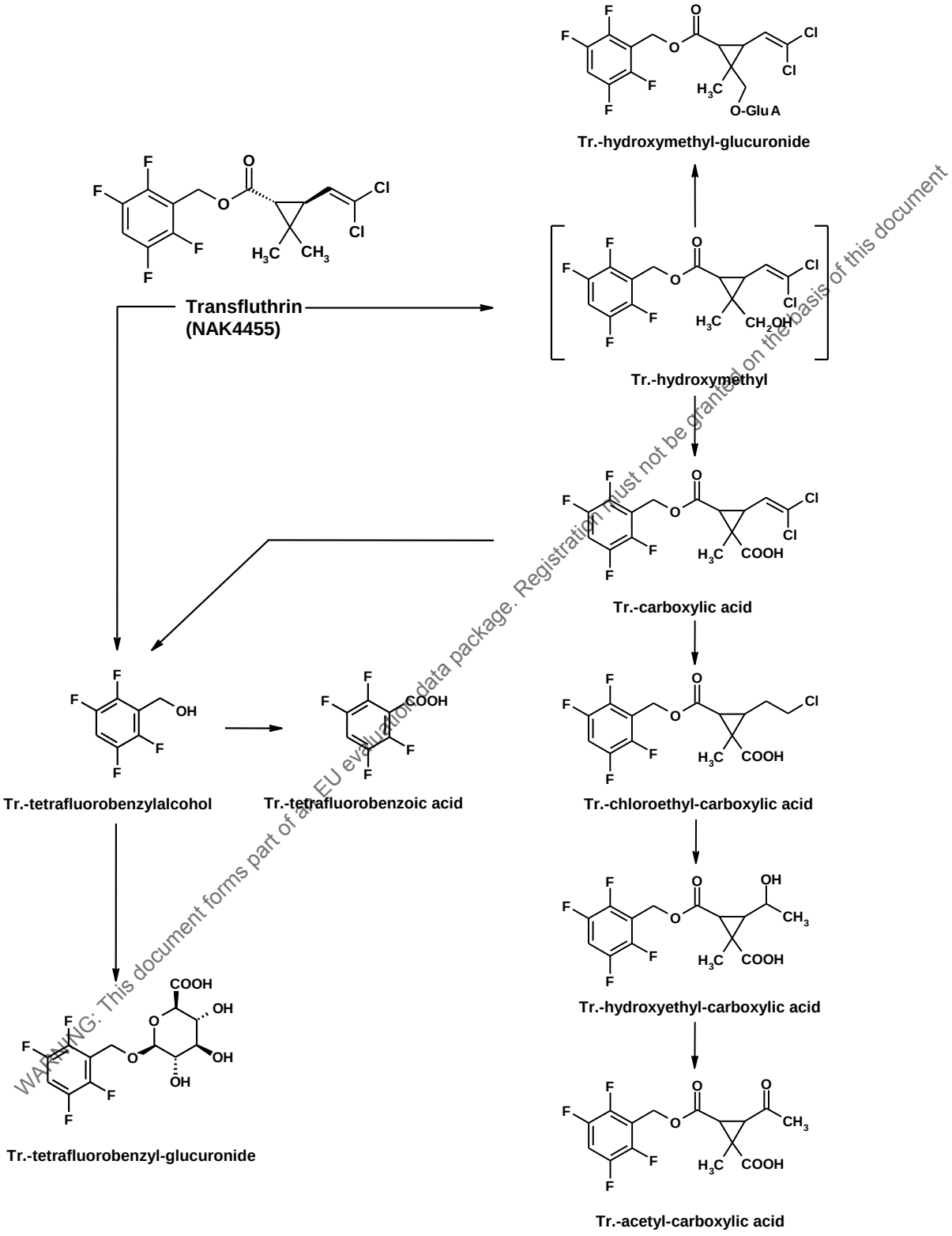


Figure A6.2/02-5: The proposed metabolic pathway of [methylene-^{14}C]Transfluthrin



[Tr. = Transfluthrin]

Table 1: Comparison of dermal penetration characteristics for pyrethroids (particularly where listed in Annex 1 to EC 91/414)

Compound name	Dermal absorption	MW	Log P_{ow}	Product[1]	Reference
Alpha-cypermethrin	10% default assumed	416.3	5.5		http://europa.eu.int/comm/food/plant/protection/evaluation/existactive/list_alpha_cypermethrin.pdf
Beta-cyfluthrin	10% default assumed	434.3	5.9	EC50	http://europa.eu.int/comm/food/plant/protection/evaluation/existactive/list1-32_en.pdf
Deltamethrin	10% default assumed	505.2	4.6	25EC	http://europa.eu.int/comm/food/plant/protection/evaluation/existactive/list1-31_en.pdf
Esfenvalerate	0.6% (human epidermis) 44% (rat skin)	419.9	6.24	EC	http://europa.eu.int/comm/food/plant/protection/evaluation/existactive/list1-15_en.pdf
Lambda-cyhalothrin	<0.3% (human, in-vivo)	449.9	7.0	5EC	http://europa.eu.int/comm/food/plant/protection/evaluation/existactive/list1-24_en.pdf
Permethrin	2% (human, in-vivo)	391.3	6.1		Ross et al (2001)
Cypermethrin	0.1 –1.8% Human in-vivo	416.3	6.6		Handbook of Pesticide Toxicology, Vol 2 p 1268; Eadsforth (1988); Woollen (1992)

1: Product: type is that which appears to be that used for Annex 1 approval, not confirmed.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 January 2007
Evaluation of applicant's justification	Considering the available data submitted by the applicant, a default dermal absorbtion of 10% is justified.
Conclusion	Applicant's justification is acceptable. A dermal absorption of 10% can be adopted for transfluthrin by default.
Remarks	No remarks
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc. IIIA

Short-term repeated dose toxicity

28-Day oral rat study

SECTION A6.3.1

BPD Data set IIA/ Annex Point VI.6.3

Official use only

	36 REFERENCE	
36.1	**Reference**	[redacted] (1990). NAK 4455. Subacute oral study of toxicity to rats. [redacted] unpublished report no. 19187[redacted], [redacted] [redacted] Report No. T 8027054 [BES Ref: MO-03-009936] Report date: June 19, 1990. Unpublished.
36.2	**Data protection**	Yes
36.2.1	Data owner	Bayer CropScience
36.2.2	Companies with letters of access	
36.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	37 GUIDELINES AND QUALITY ASSURANCE	
37.1	**Guideline study**	Yes OECD 407 Repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study (1981)
37.2	**GLP**	Yes
37.3	**Deviations**	No
	38 MATERIALS AND METHODS	
38.1	**Test material**	NAK 4455 (transfluthrin)
38.1.1	Lot/Batch number	130187
38.1.2	Specification	As given in section 2

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SECTION A6.3.1

28-Day oral rat study

BPD Data set IIA/ Annex Point VI.6.3

38.1.2.1	Description	Liquid from 50C (solid below 50C), dark brown
38.1.2.2	Purity	95.0%
38.1.2.3	Stability	Test substance was stored at room temperature in laboratory cabinet and kept stable throughout the study—Stable to December 1987. Test substance was formulated in polyethylene glycol E 400 for treatment daily. Excess was discarded. It was discovered during the test that the test substance was settling to the bottom of the container (resulting in early animals getting a lower than expected dose and later animals a higher than expected dose); thus the methodology was changed to have constant stirring during the administration period. This deviation is not thought to have had any effect on the results.
38.2	**Test Animals**	
38.2.1	Species	Rat
38.2.2	Strain	Bor:WISW (SPF-Cpb) (Wistar)
38.2.3	Source	[redacted]
38.2.4	Sex	Male and female
38.2.5	Age/weight at study initiation	7 – 8 weeks Mean weight at start of 153 g (males) and 136 g (females)
38.2.6	Number of animals per group	30 rats/sex/group (except high dose group which had 35/sex/group)
38.2.7	Control animals	Yes
38.3	**Administration/ Exposure**	Oral (gavage)
38.3.1	Duration of treatment	28 days
38.3.2	Frequency of exposure	Daily
38.3.3	Observation period	4 weeks
38.3.4	**Oral**	
38.3.4.1	Type	Gavage
38.3.4.2	Concentration	0, 10, 50 250 mg/kg bw
38.3.4.3	Vehicle	Polyethylene glycol E 400
38.3.4.4	Concentration in vehicle	0. 0.2, 1.0, 5.0 %
38.3.4.5	Total volume applied	5 mL/kg bw
38.3.4.6	Controls	Vehicle
38.4	**Examinations**	
38.4.1	Observations	

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SECTION A6.3.1

28-Day oral rat study

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38.4.1.1	Clinical signs	Observed daily.
38.4.1.2	Mortality	Observed daily.
38.4.2	Body weight	Weekly.
38.4.3	Food consumption	Calculated weekly.
38.4.4	Water consumption	No
38.4.5	Ophthalmoscopic examination	No
38.4.6	Haematology	Yes, 5 rats/sex/group at end of treatment and end of observation. Parameters: Haematocrit, haemoglobin, mean cell haemoglobin concentration, mean haemoglobin content of erythrocytes, erythrocyte count, total and differential leukocyte count, thrombocyte count, mean corpuscular volume of erythrocytes and coagulation time.
38.4.7	Clinical Chemistry	Yes, 5 rats/sex/group at end of treatment and end of observation. Parameters: glucose, total cholesterol, urea, total bilirubin, creatine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glutamate dehydrogenase, inorganic phosphate, sodium, potassium, chloride, calcium.
38.4.8	Urinalysis	Yes, 5 rats/sex/group at end of treatment and end of observation following ca. 16 hours fast. Parameters: pH, protein, glucose, blood, urobilinogen, and after sedimentation: bacteria, epithelia, erythrocytes, leukocytes, amorphous slats, triple phosphates, and calcium oxalates
38.5	**Sacrifice and pathology**	
38.5.1	Organ Weights	Yes, 10 rats/sex/group at end of treatment and end of observation. Organs: liver, kidneys, adrenals, testes, ovaries, thymus, spleen, brain, heart, lung and thyroid
38.5.2	Gross and histopathology	Yes, 10 rats/sex/group at end of treatment and end of observation and animals dying during study if autolysis had not set in. Histopathology was performed on all organs listed here. The following organs were collected and preserved in 10% aqueous formaldehyde solution from 5 rats/sex/group: brain, pituitary, thyroid, thymus, stomach, intestine (4 locations), liver, pancreas, kidneys, adrenals, urinary bladder, spleen, heart, lungs, uterus, lymph nodes (mandibular and mesenteric), bones (sternum and femur), testes, ovaries, epididymis, ears, and skeletal muscle. Bone marrow smears were prepared. **Specific neurohistopathological investigation:** The following organs were collected and preserved in 10% formaldehyde from 5 rats/sex/group after in-situ perfusion fixation: eyes, brain, muscle, sciatic nerve, ears, spinal marrow.
38.5.3	Other examinations	Hepatic enzyme induction: from 5 rats/sex/group at end of treatment and end of observation samples of liver were collected and frozen at

		necropsy, then were examined for enzyme induction and triglyceride levels, specifically: N-demethylase, O-demethylase, cytochrome P450 and triglycerides.
38.5.4	Statistics	Arithmetic group means, standard deviations, and for the organ weights, the upper and lower confidence limits, were calculated for body weights, clinical chemistry, and organ weights. Data for test animals was compared to data for control animals using the Mann-Whitney U and Wilcoxon tests. Differences were considered significant at the 5% and 1% probability levels.
38.6	**Further remarks**	None
		39 RESULTS AND DISCUSSION
39.1	**Observations**	
39.1.1	Clinical signs	Tremor was seen among animals of the top dose level (250 mg/kg bw) during the early part of the study. Incidence was highest (25/35 males, 22/35 females) during the first 9 days, however in some cases it continued into the third and forth week. In each case, tremors began 4 – 7 hours post administration and had resolved by the next day. Additionally, two female rats in the high dose group had seizures. There was no evidence of tremor or seizure among rats dosed with 50 mg/kg bw or below. There were no clinical findings in animals in any dose group during the observation period (4 weeks post treatment).
39.1.2	Mortality	In the 250 mg/kg bw group, 2 males and 5 females died on the 2nd and 3rd days – tremors had been observed in all animals that died except one female; additionally seizures had been observed in two females. There were no mortalities in rats dosed with 50 mg/kg bw or below. There were no deaths in animals in any dose group during the observation period (4 weeks post treatment).
39.2	**Body weight gain**	No significant effect was seen in body weight gain in the male rats. In the female rat 10 mg/kg bw dose group, animals were slightly but significantly lighter than controls. Because this group was significantly lighter than controls at the start of the study and because the effect was not seen in higher dose groups, this was not considered to be a treatment related effect. No treatment related effects were seen on body weight during the observation period (4 weeks post treatment). The females in the 10 mg/kg bw dose group continued to have significantly lower bodyweight, likely due to reduced initial bodyweight.
39.3	**Food consumption and compound intake**	No effect was seen on food consumption. No effect was seen on food consumption during the observation period (4 weeks post treatment).
39.4	**Ophthalmoscopic examination**	Not applicable

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28-Day oral rat study

BPD Data set IIA/ Annex Point VI.6.3

39.5	**Blood analysis**	
39.5.1	Haematology	There were no toxicologically significant changes in haematology. In the males 250 mg/kg bw dose group the leukocyte count and mean cell haemoglobin concentration were slightly but significantly higher than controls and coagulation time slightly but significantly shorter. As changes were within normal physiological parameters, these were judged not to be of toxicological significance. Males in the 10 mg/kg bw dose group had a slightly but significantly elevated erythrocyte count. No other haematological effects were observed. At the end of the observation period, no haematological changes considered to be of any toxicological relevance were observed.
39.5.2	Clinical chemistry	There were no toxicologically significant changes in clinical chemistry. The statistically significant changes observed (glutamate dehydrogenase, chloride, potassium, calcium levels and creatinine) were within normal physiologic ranges and thus were not considered of any toxicological relevance. At the end of the observation period, male rats in the 10 mg/kg bw group had slightly but significantly increased alkaline phosphatase, and males in the 50 and 250 mg/kg bw group had a decrease in creatine and sodium levels. Females in all treatment groups had an increase in calcium.
39.5.3	Urinalysis	There were no toxicologically significant changes uncovered by urinalysis. Some animals had epithelial cells in the urine—this is not uncommon and did not appear to be of toxicological significance. At the end of the observation period (4 weeks post treatment) some animals had epithelial cells in the urine.
39.6	**Sacrifice and pathology**	
39.6.1	Organ weights	The absolute and relative liver weights of males and females in the 250 mg/kg bw group were significantly elevated over controls. Also in the high dose group, the males had elevated thyroid weights (absolute and relative) and elevated kidney weights (relative) and the females had elevated kidney weights (absolute and relative). In the 50 mg/kg bw dose group, males had elevated brain weights (absolute). In the 10 mg/kg bw dose group, females had elevated kidney weights (relative). In animals sacrificed at the end of the observation period (4 weeks post treatment), there was no difference in organ weights between treated animals and controls.
39.6.2	Gross and histopathology	In the animals that died spontaneously during the study, the following were noted: patchy colouring or mottling of lung, spleen, kidneys, distended and/or fluid filled lung, reddish mucous in small intestine. Histopathologically, slight congestion and haemorrhage of the lung were noted. In two cases, focal alveolar emphysema and in one case alveolar oedema. In animals sacrificed at end of treatment, patchy thymus was noted in three animals (including one control), and mottled kidneys in one

		animal. Histopathologically, minimal non treatment related changes occurred, including: alterations in the heart, lungs, kidneys, liver, urinary bladder, adrenals, ovaries and intestine—none of which appeared to be toxicologically relevant as control animals were equally affected. In animals sacrificed at the end of the observation period, the following gross changes were noted: mottled kidneys, spleen colour changed, and patchy thymus; each in one animal. No animals were examined histopatholgoically.
39.7	**Other**	Enzyme induction: No changes were seen in treated females. Male animals in the 250 mg/kg bw dose group had slightly but significantly elevated O-demethylase activity—however, individual results were within normal physiologic range. At the end of the observation period, no changes were seen in treated females. Male animals in the 250 mg/kg bw dose group had slightly but significantly elevated N-demethylase activity—however, individual results were within normal physiologic range.
	40	**APPLICANT'S SUMMARY AND CONCLUSION**
40.1	**Materials and methods**	Groups of 30 – 35 male and 30 – 35 female Bor: WOSW (SPF-Cpb) rats were given NAK 4455 for four weeks by gavage at doses of 0, 10, 50 ad 250 mg/kg bw. Fifteen rats/sex/group were sacrificed at the end of the treatment period and the remainder were observed for a subsequent 4 weeks and then sacrificed. Five sacrificed animals/sex/group were used for haematology, clinical chemistry, urinalysis and liver enzyme induction, 5 for gross and histopathology of major organs, and 5 for examination of nerves and nerve tissue. This study fulfils requirements of OECD 407 (1981), exceeds guideline requirements in the number of animals per group, and provides a technically very competent evaluation of toxicity to rats over 28 days.
40.2	**Results and discussion**	The principal findings attributed to NAK-4455 were transient appearance of tremor, resolving on discontinuation of exposure, seizures in two animals, and the death of 7 animals after previous tremors in the high dose group. These findings are typical of other pyrethroids and were not seen in groups receiving lower doses. No treatment related effects were seen on body weight or food consumption. More subtle changes included a transient decrease in clotting time in males in the high dose group, a transient increase in liver weight in males and females in the top dose group and liver enzyme induction (O-demethylase followed by N-demethylase) in males in the high dose group. Kidneys in males and females were also transiently increased in weight. The urinalysis did not reveal any particular concerns, however, after sedimentation urine of both sexes was found to contain epithelial cells. Other statistically significant changes in single parameters, lacking

		supporting biological evidence of change in associated parameters including histopathology, were not considered to be of toxicological relevance. The lowest effect level in this study was 250 mg/kg bw, based on tremor in both sexes, the no observable adverse effect level in this study was 50 mg/kg bw. Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is deemed necessary.
40.3	**Conclusion**	
40.3.1	LO(A)EL	LOAEL was 250 mg/kg bw based on tremor (and death) in both sexes.
40.3.2	NO(A)EL	NOAEL was 50 mg/kg bw in both sexes.
40.3.3	Other	This short but powerful study demonstrated the principal effect of transfluthrin to be evident as post-dosing tremors and death, without pathological correlate even in animals which died. Animals surviving tremors appeared to recover completely.
40.3.4	Reliability	1
40.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	1 March 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted. It is noted that the tremors, observed in the high dose animals, occurred in the early part of the study, and were observed 4-7 h post administration, indicating that this is an acute effect of transfluthrin.
Conclusion	LO(A)EL: 250 mg/kg bw/day on the basis of tremors, seizures and mortality, and increased relative liver weight (17-20%) NO(A)EL: 50 mg/kg bw/day 22-3-2011: There is a significant difference between both 50 mg/kg bw/day and control as well as 100 mg/kg bw/day and control on day 56 but no evident doses effect relation.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3.1-1. Main findings observed after a 4-week treatment period of NAK 4455 in male and female rats

General parameters	Males				Females			
Dose, mg/kg bw	**0**	**10**	**50**	**250**	**0**	**10**	**50**	**250**
Bodyweight, g								
Day 7	185	186	185	185	148	142**	148	148
Day 28	248	256	254	248	171	165*	169	170
Day 56	284	292	295	291	185	176*	184	183
Tremors (no. of animal having tremors at least once/no. of animals)								
Week 1	0	0	0	20/30	0	0	0	9/30
Week 2	0	0	0	2/33	0	0	0	7/30
Week 3	0	0	0	5/33	0	0	0	7/30
Week 4	0	0	0	9/33	0	0	0	12/30
Week 5 – 8	0	0	0	0	0	0	0	0
Seizures (no. of animal having seizures/no. of animals)								
Week 1	0	0	0	0	0	0	0	2/30
Week 2 – 8	0	0	0	0	0	0	0	0
Mortality (no. of animals dying/no. of animals)								
Week 1	0	0	0	2/30	0	0	0	5/30
Week 2 – 8	0	0	0	0	0	0	0	0
Haematology								
Dose, mg/kg bw	**0**	**10**	**50**	**250**	**0**	**10**	**50**	**250**
Erythrocytes, 10^{12}/L								
Day 28	8.24	8.94*	8.14	8.34	8.04	8.07	8.12	8.09
Day 56	8.71	8.76	9.08	9.00	8.07	8.63	8.73**	8.49*
Leukocytes, 10^9/L								
Day 28	4.1	6.0	5.4	6.4**	5.4	5.5	5.2	5.0
Day 56	4.5	3.0*	4.7	5.6	4.1	4.0	4.6	3.2*
Haemoglobin, g/L								
Day 28	154	160	151	155	148	145	148	145
Day 56	152	154	155	152	144	153*	153*	148
Haematocrit, L/L								
Day 28	0.469	0.489	0.445	0.445	0.447	0.438	0.443	0.436
Day 56	0.455	0.457	0.461	0.453	0.432	0.461*	0.467*	0.448

Mean cell Hb conc., g/L erythrocyte								
Day 28	330	327	341	342*	331	330	335	333
Day 56	334	337	338	336	334	332	327	331
Hepatoquick, sec								
Day 28	33.1	29.8	30.9	28.2**	30.0	28.8	30.0	33.2
Day 56	33.1	34.2	34.1	34.8	32.5	31.3	31.3	30.5
Clinical chemistry								
Dose, mg/kg bw	**0**	**10**	**50**	**250**	**0**	**10**	**50**	**250**
Alkaline phosphatase, U/L								
Day 28	370	396	350	371	207	226	212	221
Day 56	217	296**	274	254	190	195	171	180
Glutamate dehydrogenase, U/L								
Day 28	2.3	4.3*	3.3	3.4	3.4	3.4	2.6	3.3
Day 56	2.1	0.8	3.0	2.3	2.5	4.2	3.5	3.3
Creatinine, µM								
Day 28	60	58	52	54	64	52*	52**	46**
Day 56	52	49	44*	44**	42	46	45	41
Sodium, mM								
Day 28	147	148	147	146	145	145	146	145
Day 56	145	145	144*	144*	144	144	145	144
Potassium, mM								
Day 28	6.0	5.8	5.4*	5.8	5.2	4.8	5.3	5.4
Day 56	6.0	5.2	5.8	5.3	4.8	4.9	5.1	4.8
Calcium, mM								
Day 28	2.73	2.77	2.71	2.71	2.59	2.56	2.63	2.70*
Day 56	2.67	2.65	2.67	2.68	2.53	2.60*	2.68**	2.69**
Chloride, mM								
Day 28	98	100	101*	98	100	101	102	99
Day 56	100	101	99	100	102	102	102	102
Enzyme induction								
N-demethylase, mU/g								
Day 28	110.8	126.4	127.9	126.1	51.4	50.0	49.1	51.0
Day 56	100.2	127.0*	123.5	153.3**	53.5	50.4	59.2	49.9
O-demethylase, mU/g								
Day 28	8.5	10.1	9.2	11.5*	7.8	7.9	7.2	6.9
Day 56	8.8	10.0	9.4	9.8	7.5	7.2	7.9	7.1

Organ weights (day 28)								
Absolute, mg								
Thyroid	10	11	12	12*	9	10	10	10
Liver	10724	11044	11138	12312*	6481	6697	6859	8213*
Kidney	1716	1795	1751	1800	1144	1183	1171	1251*
Brain	1735	1739	1810*	1706	1627	1612	1595	1615
Relative, mg/100g								
Thyroid	4	4	5	5*	5	6	6	6
Liver	4276	4205	4342	5021*	3980	3947	4039	4766*
Kidney	684	684	684	736*	667	697*	690	726*

*p < 0.05 compared to controls

**p < 0.01 compared to controls

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	41 REFERENCE	
41.1	**Reference**	[redacted] (1990). NAK 4455. Subacute dermal study of toxicity to rabbits. [redacted] [redacted] Report No. T 5029590 [BES Ref: MO-03-009897] Report date: July 12, 1990 Unpublished
41.2	**Data protection**	Yes
41.2.1	Data owner	Bayer CropScience
41.2.2	Companies with letters of access	
41.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	42 GUIDELINES AND QUALITY ASSURANCE	
42.1	**Guideline study**	Yes OECD 410 Repeated Dose Dermal Toxicity Rodent: 21/28-day Study (1981) EPA FIFRA § 82-2 Repeated Dose Dermal Toxicity: 21 day study (1982)
42.2	**GLP**	Yes
42.3	**Deviations**	No
	43 MATERIALS AND METHODS	
43.1	**Test material**	NAK 4455 techn. (transfluthrin)
43.1.1	Lot/Batch number	250987
43.1.2	Specification	As given in section 2

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43.1.2.1	Description	Liquid from 50C (solid below 50C), dark brown
43.1.2.2	Purity	95.0%
43.1.2.3	Stability	Test substance was stored at room temperature in laboratory cabinet and kept stable throughout the study—Stable to October 1988. Test substance was formulated before each treatment with Cremophor EL (2% v/v) in sterile physiological saline. During application formulation was kept homogenous on a magnetic stirrer. Stability confirmed by analysis.
43.2	**Test Animals**	
43.2.1	Species	Rabbit
43.2.2	Strain	HC:NZW (New Zealand White)
43.2.3	Source	[redacted]
43.2.4	Sex	Male and female
43.2.5	Age/weight at study initiation	10 – 16 weeks Mean weight range at start of study 2.62 – 3.10 kg (males) and 2.52 – 3.27 kg (females)
43.2.6	Number of animals per group	5 rabbits/sex/group (except control and high dose group which had 10/sex/group)
43.2.7	Control animals	Yes
43.3	**Administration/ Exposure**	Dermal
43.3.1	Study design	

Group	Dose, mg/kg bw	% formulation
Control group	0	0
Low dose group	20	1
Mid dose group	200	10
High dose group	1000	50

43.3.2	Duration of treatment	21 days
43.3.3	Frequency of exposure	5 days per week
43.3.4	Observation period	14 days
43.3.5	**Dermal**	

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43.3.5.1	Area covered	>10 % of body surface
43.3.5.2	Occlusion	Semiocclusive
43.3.5.3	Vehicle	Cremophor EL (2% v/v) in physiological saline
43.3.5.4	Concentration in vehicle	0, 1, 10, 50 %
43.3.5.5	Total volume applied	2 mL/kg bw
43.3.5.6	Duration of exposure	6 h
43.3.5.7	Removal of test substance	After exposure, treatment area cleaned with soap and water.
43.3.5.8	Controls	Vehicle
43.4	**Examinations**	
43.4.1	Observations	
43.4.1.1	Clinical signs	Yes, observed daily.
43.4.1.2	Mortality	Yes, observed daily.
43.4.2	Body weight	Yes, weekly.
43.4.3	Food consumption	Yes, calculated weekly.
43.4.4	Water consumption	No
43.4.5	Ophthalmoscopic examination	No
43.4.6	Haematology	Yes, all rabbits before start of study, at end of treatment and end of observation. Parameters: Haematocrit, haemoglobin, mean cell haemoglobin concentration, mean haemoglobin content of erythrocytes, erythrocyte count, total and differential leukocyte count, thrombocyte count, mean corpuscular volume of erythrocytes and coagulation time.
43.4.7	Clinical Chemistry	Yes, all rabbits before start of study, at end of treatment and end of observation. Parameters: glucose, total cholesterol, urea, total bilirubin, creatine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, inorganic phosphate, sodium, potassium, chloride, calcium.
43.4.8	Urinalysis	Not performed
43.5	**Sacrifice and pathology**	
43.5.1	Organ Weights	Yes, all rabbits following autopsy (1 or 2 days after last treatment or following observation period). Organs: liver, kidneys, adrenals, testes, ovaries, spleen, brain, heart , lung and thyroid

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43.5.2	Gross and histopathology	Yes, all rabbits following autopsy (1 or 2 days after last treatment or following observation period). The following organs were collected for gross examination: treated and untreated skin, thyroid, heart, lung, liver, kidneys, spleen, adrenals, testicles, epididymis, ovaries, uterus and sternum. All the organs were fixed in Bouin's solution. In addition parts of liver and kidney were fixed in 10 % formalin calcium. The following organs were examined histopathologically: liver, lung, spleen, heart, kidney, adrenals (2x), thyroid (2x), testicles (2x), epididymis (2x), uterus (3x), sternum, ovaries (2x), and untreated (skin sample 1) and treated (skin sample 2) skin.
43.5.3	Other examinations	Skin: Treated skin was examined for skin toleration by examining for redness (before start of study and 24 hours after each treatment) and scored according to Draize and skin fold thickness in centre of exposure area was measured (before start and on days 3, 8, 10, 14, 16 and 21). Enzyme induction: All rabbits following autopsy were examined for enzyme induction and triglyceride levels in the liver, specifically: N-demethylase, O-demethylase, cytochrome P450 and triglycerides.
43.5.4	Statistics	Arithmetic group means, standard deviations, and for the organ weights, the upper and lower confidence limits, were calculated for body weights, clinical chemistry, and organ weights. Data for test animals was compared to data for control animals using the Mann-Whitney U and Wilcoxon tests. Differences were considered significant at the 5% and 1% probability levels.
43.6	**Further remarks**	
		44 RESULTS AND DISCUSSION
44.1	**Observations**	
44.1.1	Clinical signs	No effects
44.1.2	Mortality	No mortalities at any dose
44.2	**Body weight gain**	No treatment related effects were observed (males at 1000 mg/kg/d had slightly but significantly reduced weight as compared to controls on day 30 which remained within the normal physiological range).
44.3	**Food consumption and compound intake**	No treatment related effects were seen. Males in the high dose group were slightly but significantly lighter on day 30.
44.4	**Ophthalmoscopic examination**	Not applicable
44.5	**Blood analysis**	
44.5.1	Haematology	There were no toxicologically significant changes in haematology. Males in the 20 mg/kg bw group had slightly but significantly decreased MCH on day 23, as did females in the 200 mg/kg bw group.

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44.5.2	Clinical chemistry	No effects
44.5.3	Urinalysis	Not applicable
44.6	**Sacrifice and pathology**	
44.6.1	Organ weights	There were no toxicologically significant changes in organ weights. Males in the 20 mg/kg bw group had slightly but significantly increased relative kidney weights. As this effect was not dose-related, it was probably due to Nosema cuniculi infestation and was not considered of any toxicological relevance.
44.6.2	Gross and histopathology	The vast majority of gross pathology changes were localized to the kidneys and included changes in colour and surface. This did not appear to be a dose-related response as it occurred randomly throughout the groups (including controls), and may be due to Nosema cuniculi infestation. Additionally, colour change on the lung was noted in 3 animals, on the spleen in 2 animals, on the liver in 1 animal. One animal had a cyst on its lung and another had emphysema. Histopathology revealed a treatment related effect on skin in all of the animals in the 1000 mg/kg bw dose group and in 7/10 of the animals in the 200 mg/kg bw dose group. Effects included thickening of the epidermis, hyperkeratosis, and in one animal, suppurative dermatitis. No effects were seen in animals in the 20 mg/kg bw dose group. Nor were effects noted in the 1000 mg/kg bw dose group 2 weeks after final treatment, suggesting that skin damage is transient. No other organs showed treatment related effects—nephropathy, lung cell infiltration and liver cell necrosis occurred randomly throughout groups (including controls).
44.7	**Other**	Skin: Slight to moderate redness was noted in the 200 and 1000 mg/kg bw dose groups up to day 12. As treatment progressed, scaling, encrustation, swelling and red patches were noted in these groups, as was increased skin fold thickness. Note that in table A6.3.2-1 "necrosis" is listed as an endpoint for skin evaluation. This is reported as "necrosis" because it was reported as "necrosis" in applicant's study, however it is almost certainly not necrosis as histopathological examination of the skin did not reveal necrosis nor did concurrent Draize scoring suggest necrosis. Enzyme induction: N-demethylase was induced in the male 200 and 1000 mg/kg bw dose groups and the female 200 mg/kg bw dose group at end of treatment (days 23/34); no induction was seen at the end of the observation period (day 38). Induction was not correlated with dose, showed large variability was within normal physiologic parameters, thus was not concluded to have toxicological significance. No induction was seen in other dose groups.

45 APPLICANT'S SUMMARY AND CONCLUSION

45.1	**Materials and methods**	Groups of 5 – 10 male and 5 – 10 female HC: NZW rabbits were dermally dosed with NAK 4455 for 21 days (5 days/week) at doses of 0, 20, 200 and 1000 mg/kg bw. Five rats/sex/group were sacrificed at the

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		end of the treatment period and the remainder were observed for a subsequent 14 days and then sacrificed. Haematology, clinical chemistry, skin tolerance and liver enzyme induction were performed for all animals as was gross and histopathology. This study fulfils requirements of OECD 410 (1981), with the minor deviation that clotting time was not measured, and provides a technically very competent evaluation of toxicity to rabbits over 21 days.
45.2	**Results and discussion**	The principal finding attributed to NAK-4455 was reddening of the skin. No systemic effects were noted. No treatment related effects were seen on body weight or feed consumption. No treatment effects were seen on haematology, clinical chemistry, enzyme induction, gross or histopathology. Statistically significant changes in single parameters, lacking supporting biological evidence of change in associated parameters including histopathology, were not considered to be of toxicological relevance. At 1000 mg/kg bw/day, only minor localised effects at the skin application site were found. Based on this study, a lowest observed adverse effect level for systemic effects was in excess of the highest dose tested (upper limit dose). The no observed adverse effect level for systemic effects is thus 1000 mg/kg bw for both sexes based on this study. Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is deemed necessary.
45.3	**Conclusion**	
45.3.1	LO(A)EL	No systemic effects were seen, thus no LOAEL was established.
45.3.2	NO(A)EL	NOAEL (systemic) was 1000 mg/kg bw in both sexes.
45.3.3	Other	
45.3.4	Reliability	1
45.3.5	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	13-03-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	The version of the applicant is adopted
Conclusion	NOAEL systemic: 1000 mg/kg bw/day (highest dose tested) NOAEL local: 20 mg/kg bw/day, on the basis of redness, scaling, encrustation, swelling, red patches, increased skin fold thickness, thickening of the epidermis, and hyperkeratosis.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_3.2-1. Main findings observed in male and female rabbits after subacute dermal exposure with transfluthrin

Parameter	0 mg/kg bw		20 mg/kg bw		200 mg/kg bw		1000 mg/kg bw		dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	10	10	5	5	5	5	10	10		
Enzyme induction (N-demethylase)	126.5	134.9	135.1	169.5	164.4*	183.2**	146.7*	161.8	-	-
Skin										
redness	0	0	0	0	2/5	4/5	10/10	8/10	+	+
scaling	0	0	0	0	5/5	5/5	10/10	10/10	+	+
red/swollen	0	0	0	0	2/5	0/5	8/10	9/10		
necrotic †	0	0	0	0	0	0	1/10	6/10		
increased skin fold thickness			2/5	1/5	4/5	3/5	7/10	7/10	+	+
Skin histopathology (post treatment)										
Thickening epidermis	0/5	0/5	0/5	0/5	4/5	3/5	5/5	5/5	+	+
Hyperkeratosis	0/5	0/5	0/5	0/5	0/5	2/5	2/5	5/5	+	+
Suppurative dermatitis	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5		

0 = no response, *p < 0.05 as compared to controls, **p < 0.01 as compared to controls, †Note that this is not actual "necrosis," for a more complete explanation, please see results section.

Table A6_3.2-2. Skin redness scores observed in male and female rabbits after a subacute dermal exposure with transfluthrin

	Mean redness score (Draize)													
Male	Day													
Dose, mg/kg bw	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13-23**
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	0	0	0.2	0.4	0	0	0	0	0	0	0	0	0	0
1000	0	0.3	0.7	1.3	1.1	0.7	0.2	0	0.1	0.2	0.2	0.2	0.2	0
Female	Day													

Dose, mg/kg bw	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13-23**
0	0	0	0	0	0	0	0	0	0	0	0	0.1	0.1	0
20	0	0	0	0	0	0	0	0	0	0	0	0	0	0
200	0	0	0.2	0.4	1.2	0.4	0	0	0	0	0	0	0	0
1000	0	0	0.3	0.8	0.4	0	0	0.3	0.1	0.1	0.1	0.1	0	0

0 = No response

Doc. IIIA **SECTION A6.3.3** **BPD Data set IIA/ Annex Point VI.6.3**	**Repeated dose toxicity (inhalation)**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	**Available data on repeated dose inhalation toxicity (28 day) are not considered to be key studies as data on sub-chronic inhalation (90 day) are available.**	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	13-03-2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable	
Conclusion	The justification of the applicant is acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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Subchronic toxicity

18-Week oral rat study

Official use only

	46 REFERENCE	
46.1	**Reference**	[redacted] (1990) NAK 4455. Subchronic toxicological study in rats (Administration in diet for up to 18 weeks). [redacted] [redacted] Report no. T9024986 [BES Ref: MO-03-009872] Report date: November 30, 1990 Unpublished
46.2	**Data protection**	Yes
46.2.1	Data owner	Bayer CropScience
46.2.2	Companies with letters of access	
46.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	47 GUIDELINES AND QUALITY ASSURANCE	
47.1	**Guideline study**	Yes US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)
47.2	**GLP**	Yes
47.3	**Deviations**	No
	48 MATERIALS AND METHODS	
48.1	**Test material**	NAK 4455 (transfluthrin)
48.1.1	Lot/Batch number	130187
48.1.2	Specification	As given in section 2
48.1.2.1	Description	Liquid from 50ºC (solid below 50ºC), dark brown
48.1.2.2	Purity	95.0%
48.1.2.3	Stability	Verified by analysis during the study.
48.2	**Test Animals**	
48.2.1	Species	Rat
48.2.2	Strain	Bor:WISW (SPF-Cpb) (Wistar)
48.2.3	Source	[redacted]

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48.2.4	Sex	Male and female
48.2.5	Age/weight at study initiation	6 weeks Weight range at start of study 95-116 g (males) and 89-113 g (females)
48.2.6	Number of animals per group	10 rats/sex/group (except control and 5000 ppm groups which had an additional 10 animals/sex/group—"satellite groups")
48.2.7	Control animals	Yes
48.3	**Administration/ Exposure**	Oral (diet)
48.3.1	Duration of treatment	95-97 days (plus a 4-week exposure for satellite groups, for duration of 126-127 days)
48.3.2	Frequency of exposure	Continuous (diet, *ad-lib*)
48.3.3	Postexposure period	None
48.3.4	**Oral**	
48.3.4.1	Type	In food
48.3.4.2	Concentration	In food 0, 10, 50, 500, and 5000 ppm equivalent to Males: 0, 0.8, 3.5, 37.5 and 384.1 (397.2 in satellite group) mg/kg bw Females: 0, 0.9, 4.4, 47.3 and 515.4 (487.5 in satellite group) mg/kg bw
48.3.4.3	Vehicle	Test compound mixed with 1% peanut oil then food powder
48.3.4.4	Concentration in vehicle	95%
48.3.4.5	Total volume applied	Not applicable
48.3.4.6	Controls	Diet with 1% peanut oil
48.4	**Examinations**	
48.4.1	Observations	
48.4.1.1	Clinical signs	Yes, observed twice daily.
48.4.1.2	Mortality	Yes, observed twice daily.
48.4.2	Body weight	Yes, weekly.
48.4.3	Food consumption	Yes, calculated weekly.
48.4.4	Water consumption	Yes, calculated weekly.
48.4.5	Ophthalmoscopic examination	Yes, at start of study and in 13th study week and after 17 wks (satellite groups), animals in 0 and 5000 ppm groups—surroundings of the eyes and the anterior eye sections were examined for alterations, pupil reflex test was made in a darkened room, transparent eye media and eye fundus were examined after pupil dilation with an indirect ophthalmoscope.

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48.4.6	Haematology	Yes, after approximately 5, 13 and 17 (satellite groups) weeks. Parameters: Haematocrit, haemoglobin, mean cell haemoglobin concentration, mean haemoglobin content of erythrocytes, erythrocyte count, total and differential leukocyte count, erythrocyte morphology, thrombocyte count, mean corpuscular volume of erythrocytes and thromboplastin time.
48.4.7	Clinical Chemistry	Yes, after approximately 5, 13 and 17 (satellite groups) weeks. Parameters: glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glutamate dehydrogenase, anorganic phosphate, : sodium, potassium, chloride, calcium, triglycerides, albumin, fluoride (in bones and teeth).
48.4.8	Urinalysis	Yes, after approximately 5, 13 and 17 (satellite groups) weeks following ca. 16 hours fast. Parameters: volume, specific gravity, sodium, potassium, calcium, anorganic phosphate, chloride, protein, glucose, blood, ketone bodies, bilirubin, urobilinogen, and after sedimentation: bacteria, epithelia, erythrocytes, leukocytes, cylinders and crystals
48.5	**Sacrifice and pathology**	
48.5.1	Organ Weights	Yes, at end of 13 weeks and end of 18 weeks (satellite groups) all surviving animals were sacrificed and the following organs were weighed: liver, kidneys, adrenals, testes, spleen, brain, heart, and lung.
48.5.2	Gross and histopathology	Yes, at end of 13 and 18 weeks (satellite groups) all surviving animals were sacrificed and the following organs subjected to gross pathology (Animals dying spontaneously or becoming moribund and sacrificed during treatment were autopsied as soon as possible and organs subjected to gross pathology if not autolysed): aorta, eyes, eyelids, cecum, colon, duodenum, extra-orbital lachrymal glands, brain, Harderian gland, ureter, urethra, skin, hypophysis, larynx, ileum, bone marrow (femur, sternum), mammary glands, musculature (thigh), sciatic nerve, optic nerve, oesophagus, prostate, rectum, seminal vesicle, salivary glands, sternum, trachea, vagina, spinal column, marrow (cervical, lumbar, thoracic), tongue, thyroid, thymus, stomach, liver, pancreas, kidneys, adrenals, urinary bladder, spleen, heart, lungs, uterus, lymph nodes (cervical and mesenteric), femur, testes, ovaries, epididymis, ears, and skeletal muscle. All organs were preserved in Bouin's solution. Five micron tissue sections stained with haematoxylin and eosin were prepared by Bayer. Tissues from all rats killed after 13 weeks of treatment were examined by the Pathologist and subsequently sections of liver, kidneys and thyroid gland from the animals killed after 18 weeks of treatment were examined. Sections of thyroid gland and kidneys were stained with Periodic Acid Schiff and sections of kidneys were also stained using Perl's method.
48.5.3	Other examinations	Enzyme induction: At sacrifice (13 weeks or 19 weeks for satellite groups) animals were examined for enzyme induction in the liver,

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		specifically: N-demethylase, O-demethylase, cytochrome P450 and carnitine acyl transferase.
48.5.4	Statistics	Arithmetic group means, standard deviations, and for the organ weights, differential blood counts, and fluoride concentrations, the upper and lower confidence limits, were calculated for body weights, clinical chemistry, and organ weights. Data for test animals was compared to data for control animals using the Mann-Whitney U and Wilcoxon tests. Differences were considered significant at the 5% and 1% probability levels.
48.6	**Further remarks**	
		49 RESULTS AND DISCUSSION
49.1	**Observations**	
49.1.1	Clinical signs	No effects
49.1.2	Mortality	No treatment related mortalities were seen.
49.2	**Body weight gain**	No effects
49.3	**Food consumption and compound intake**	No treatment related effects were seen on food consumption or compound intake. Males in the high dose group (both main and satellite) had slightly but significantly increased water consumption.
49.4	**Ophthalmoscopic examination**	No effects
49.5	**Blood analysis**	
49.5.1	Haematology	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and were within normal physiologic parameters for the effect.
49.5.2	Clinical chemistry	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and were within normal physiologic parameters for the effect. Although they did not achieve statistical significance for all dose groups, cholesterol was elevated outside normal parameters in females in the high dose group (regular and satellite) at 6 weeks and in males and females in the 500 ppm and high dose groups at 13 weeks (regular and satellite) and 17 weeks (satellite only). Albumin levels were also increased outside of normal parameters in the high dose groups for both sexes at all time periods. Triglycerides were slightly but significantly lowered in the 500 and 5000 ppm dose groups for both sexes at both time points. Fluoride content in bones and teeth was significantly increased in a dose dependent manner in male and female animals—statistical significance of the increase was seen starting with the 50 ppm group.
49.5.3	Urinalysis	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose

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response and/or lack of time dependence and were within normal physiologic parameters for the effect.

No significant changes were seen in females. In males, protein levels in the urine were significantly increased in the 5000 ppm (regular and satellite) group at 13 and 17 weeks—protein was apparently increased in males in the 50 and 500 ppm groups as well, but when normalized for volume, no effect was seen. Additionally, various electrolytes were increased in the high dose group in males, including sodium and phosphate at 4-5 weeks, and sodium at 13 weeks.

49.6 Sacrifice and pathology

49.6.1 Organ weights

Absolute and relative liver and kidney weights were increased in males in the 500 and 5000 (regular and satellite) dose groups. In the females, absolute and relative lung weights were increased in the 5000 ppm (regular only) dose group, relative liver weights were increased in the 500 ppm dose group and absolute and relative liver weights were increased in the 5000 ppm (regular and satellite) dose groups, relative kidney weights were increased in the 5000 ppm (satellite only) dose group.

49.6.2 Gross and histopathology

At autopsy, enlarged liver was found in 3 males in the 500 ppm dose group, 2 males in the 5000 ppm dose group, and 5 males in the 5000 ppm satellite dose group. Various other spontaneous pathologies not related to treatment were also seen.

Centrilobular hypertrophy (liver) (minimal or moderate) was seen in most animals in the high dose group (regular and satellite); minimal centrilobular hypertrophy was seen in 8/10 males and 4/10 females in the 500 ppm group. However, this is a normal liver adaptive response and is not judged to be an adverse effect.

In the regular high dose group, degeneration of proximal convoluted tubules was noted in all animals. However, in the satellite animals—treated with 5000 ppm for 18 weeks—there was no evidence of degeneration of the proximal convoluted tubules. In the kidneys of males in the 500 and 5000 ppm groups a slightly increased number of animals with yellow granular deposits in the epithelial cells of basophilic cortical tubules was observed. However, as this effect was also observed in control animals, a relation to treatment is doubtful. The appearance of the deposits in the control and 5000 ppm satellite groups suggest this is a normal change, perhaps intensified by treatment.

Male animals in the high dose and 500 ppm group were observed to have increased hypertrophy of follicular epithelium of the thyroid. This was not observed in female animals. This effect is probably secondary to liver hypertrophy and thus not considered as a direct effect of treatment.

49.7 Other

Statistically significant increased levels of all liver enzymes were seen in both sexes in the 5000 ppm group (with the exception of P450 in the female rat).

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50 APPLICANT'S SUMMARY AND CONCLUSION

50.1 Materials and methods

Groups of 10 male and female Wistar rats were given NAK 4455 for three months in diet at doses of 0, 10, 50, 500 and 5000 ppm. An additional 10 rats/sex/group were given either 0 or 5000 ppm NAK 4455 in the diet for a total time period of 18 weeks. All rats were sacrificed at the end of their respective treatment periods. Haematology, clinical chemistry, urinalysis, liver enzyme induction, measurement of fluoride levels, and gross and histopathology were performed on all animals. This study largely fulfils requirements of US EPA FIFRA § 82-1 (1984), with the main exception that a recovery period for the high dose satellite group was not performed as the animals were accidentally dosed throughout this period. Due to the numerous other sub-chronic and chronic studies available, and the type of results observed in this study, this deficiency is not considered a critical deficiency.

50.2 Results and discussion

No treatment induced changes in behaviour, appearance, mortality, growth (weight), food or compound intake was observed. No treatment related damage to the eye was observed.

The results from the gross pathology, histopathology, urinalysis clinical chemistry and enzyme induction studies suggest that liver and kidney effects occur in both sexes exposed to 5000 ppm and may begins at 500 ppm.

In the higher dose groups, liver weights were increased, liver enzymes were induced and centrilobular hypertrophy and enlarged liver were observed. These findings are clearly related to a liver adaptative response rather than an adverse effect of the product. Additionally, triglyceride levels were decreased, cholesterol levels were increased as were albumin and alkaline phosphatase.

At 5000 ppm, increased kidney weights, increased protein in the urine and reduced sodium in the urine were observed, as was increased water consumption (males only). Degenerative alterations to the proximal tubule were also noted; however these changes were reversible, as no degeneration was seen in animals dosed with 5000 ppm for 18 weeks. Moreover, no fibrosis or scarring was seen as would be expected if damage were occurring.

A dose dependent increase in fluoride content in teeth and bones was seen starting at 50 ppm, however no effect (e.g. softening) was seen on bones examined histopathologically. Therefore, in the absence of any other related finding, the increase in fluorine content was not regarded as a toxicologically relevant nor as an adverse effect.

All males in the 500 and 5000 ppm groups showed hypertrophy of the follicular epithelium of the thyroid. This may be a secondary result of altered liver physiology.

The lowest adverse effect level in this study is 500 ppm (equivalent to approximately 40 mg/kg bw) based on liver and kidney effects in both sexes. The no observable adverse effect level is 50 ppm (equivalent to approximately 4 mg/kg bw).

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		Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is necessary.	
50.3	**Conclusion**		
50.3.1	LO(A)EL	The lowest adverse effect level in this study is 5000 ppm (450 mg/kg bw/day) based on liver and kidney damage in both sexes.	X
50.3.2	NO(A)EL	The no observable adverse effect level is 500 ppm (42 mg/kg bw/day).	X
50.3.3	Other		
50.3.4	Reliability	1	
50.3.5	Deficiencies	In this study, the animals in the satellite groups were dosed for an additional 4 weeks. The purpose of the 4-week observation undosed observation period is to allow observation of reversibility or latency. This is a not a critical deficiency in the study, although, it is likely that a number of effects (e.g. enzyme induction) would have proved to be reversible. Nonetheless, the existence of an appropriately performed 90-day oral study in a non-rodent species, 90-day inhalation study in rats, and numerous chronic studies, obviate the need to duplicate this study with an observation period.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	14-03-2007
Materials and Methods	Applicants version is accepted
Results and discussion	The main observations in the liver were: increased relative liver weight in both sexes at 500 ppm and 5000 ppm (14% and 44% in males, 17% and 28% in females), enlarged livers in males at 500 ppm and 5000 ppm (3/10 and 2/10 vs. 0/10 in controls), and centrilobular hypertrophy in both sexes at 500 ppm and 5000 ppm (8/10 and 10/10 in males vs. 0/10 in controls, 4/10 and 9/10 in females vs. 0/9 in controls). In addition, in male rats at 500 and 5000 ppm the relative kidney weight was increased (11% and 14%) and thyroid hypertrophy is noted (10/10 and 10/10 vs. 0/10 in controls). Further liver enzyme activities were increased and some clinical chemistry parameters altered. Although in the results and discussion section (5.2) the applicant determines a LOAEL and NOAEL of 500 and 50 ppm respectively, in the final conclusion the applicant determines a LO(A)EL of 5000 ppm and a NO(A)EL of 500 ppm. The RMS is of opinion that based on the magnitude of the responses observed a LO(A)EL of 500 ppm and a NO(A)EL of 50 ppm is more appropriate
Conclusion	LO(A)EL: 500 ppm (37.5 mg/kg bw/day), on the basis of increased liver weight and centrilobular hypertrophy (both sexes), increased relative kidney weight (males only) and effects on clinical chemistry parameters. NO(A)EL: 50 ppm (3.5 mg/kg bw/day)
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4.1-1. Main clinical chemistry findings observed in male and female rats after subchronic exposure to transfluthrin

Parameter changed	Unit	Controls (satellite group)			10 ppm		50 ppm		500 ppm		5000 ppm (satellite group)		
Weeks post treatment		5/6	13/14	17/18	5/6	13/14	5/6	13/14	5/6	13/14	5/6	13/14	17/18
Males													
Cholesterol	mM	2.28 (2.37)	2.48 (2.33)	2.11	2.32	2.43	2.35	2.48	2.39		2.66 (2.72)	2.73 (3.09**)	3.08**
Triglycerides	mM	1.16 (1.19)	1.71 (1.54)	1.24	0.97	1.30	0.91	1.11	0.74	1.04*	0.53** (0.94)	0.87* (1.41)	1.05
Females													
Cholesterol	mM	2.41 (2.50)	2.35 (2.20)	2.29	2.57	2.55	2.33	2.36	2.53	2.30	2.64 (2.84*)	2.62 (2.56**)	2.69*
Triglycerides	mM	0.99 (1.32)	1.40 (1.17)	0.74	1.30*	1.28	0.80	1.04	0.78*	0.97*	0.62** (0.67**)	0.62** (0.81**)	0.57

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

Table A6_4.1-2. Enzyme induction findings observed in male and female rats after subchronic exposure to transfluthrin

Parameter changed	Unit	Controls		10 ppm	50 ppm	500 ppm	5000 ppm	
Weeks post treatment		13/14	17/18	13/14	13/14	13/14	13/14	17/18
Males								
N-demethylase	mU/g	131.5	161.5	129.1	139.7	122.6	172.6*	193.6
O-demethylase	mU/g	10.6	9.2	10.8	9.0	8.8	17.5**	16.4**
P450	nmol/g	30.1	27.9	31.2	31.0	30.8	41.3**	33.8*
CAT[1]	U/g	0.30	0.68	0.34	0.33	0.42	0.50*	1.01*
Females								
N-demethylase	mU/g	65.9	71.7	71.7	51.0	60.6	80.4*	99.8+
O-demethylase	mU/g	11.1	9.2	11.4	9.3	11.5	18.3**	13.8+
P450	nmol/g	27.2	19.4	25.7	24.4	29.4	25.6	26.6+
CAT[1]	U/g	1.06	1.15	1.01	1.08	1.45*	2.63**	4.48+

[1]CAT= Carnitine acyl transferase; * = p < 0.05 ; ** = p < 0.01, + = significance not tested

Table A6_4.1-3. Main pathological findings observed in male and female rats after subchronic exposure to transfluthrin

Parameter changed	Controls (satellite)		10 ppm		50 ppm		500 ppm		5000 ppm (satellite)		Dose-response +/-	
	m	f	m	f	m	f	m	f	m	f	m	f
Number of animals examined	10 (10)	9 (10)	10	10	10	10	10	10	10 (10)	10 (10)		
Mortality	0/10 (0/10)	1/10 (0/10)	0/10	0/10	0/10	0/10	0/10	0/10	0/10 (0/10)	0/10 (0/10)		
Liver												
Relative weight, mg/100g	3439 (3074)	3479 (3651)	3477	3452	3488	3785	3935*	4082**	4939** (4712**)	4443** (4682**)	+	+
Gross pathology (enlarged)	0/10 (0/10)	0/9 (0/10)	0/10	0/10	0/10	0/10	3/10	0/10	2/10 (5/10)	0/10 (0/10)	+	-
Centrilobular hypertrophy	0/10 (0/10)	0/9 (0/10)	0/10	0/9	0/10	0/10	8/10	4/10	10/10 (10/10)	9/10 (7/10)	+	+
Kidney												
Relative organ weight, mg/100g	600 (616)	637 (638)	615	636	624	676	668**	686	686* (701**)	662 (677*)	+	-
Yellow granular deposits in basophilic tubules	6/10 (8/10)	0/9 (1/10)	5/10	0/10	7/10	0/10	9/10	1/10	9/10 (10/10)	0/10 (0/10)		
Yellow granular deposits in proximal convoluted tubules	5/10 (10/10)	1/9 (0/10)	5/10	0/9	6/10	0/10	9/10	1/10	10/10 (10/10)	10/10 (10/10)	-	-
Degeneration prox. tubule	0/10 (0/10)	0/9 (0/10)	0/10	0/9	0/10	0/10	0/10	0/10	10/10 (0/10)	10/10 (0/10)	-	-
Thyroid												
Hypertrophy	0/10 (0/10)	0/9 (0/10)	0/10	0/9	0/10	0/10	10/10	0/10	10/10 (3/10)	0/10 (0/10)	-	-

- = no difference from control,* = $p < 0.05$; ** = $p < 0.01$

Doc IIIA

Repeated dose toxicity

3-month oral dog study

SECTION A 6.4.1/02

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			Official use only
		51 REFERENCE	
51.1	**Reference**	[redacted] (1989); 13-week oral toxicity (feeding) study with NAK 4455 Tech. in the dog. [redacted] unpublished report no. 085151, 6 April 1989. [redacted] d. [redacted] Report no: T 9025011. [BES Ref: MO-03-009770] Report date: 06 April 1989 Unpublished.	
51.2	**Data protection**	Yes	
51.2.1	Data owner	Bayer CropScience	
51.2.2			
51.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I	
		52 GUIDELINES AND QUALITY ASSURANCE	
52.1	**Guideline study**	Yes OECD Guidelines 409 (1981) US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)	
52.2	**GLP**	Yes	
52.3	**Deviations**	No	
		53 MATERIALS AND METHODS	
53.1	**Test material**	NAK 4455 (transfluthrin)	
53.1.1	Lot/Batch number	250987	
53.1.2	Specification	As given in section 2	
53.1.2.1	Description	Solid	
53.1.2.2	Purity	94.5%	
53.1.2.3	Stability	Stability and homogeneity and content of test article in feed were verified by analysis before beginning of study. Homogeneity and content were verified monthly during study.	
53.2	**Test Animals**		
53.2.1	Species	Dog	

Doc IIIA Repeated dose toxicity

3-month oral dog study

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53.2.2	Strain	Beagle
53.2.3	Source	[redacted]
53.2.4	Sex	Male and female
53.2.5	Age/weight at study initiation	4-6 months Weight range at delivery 6.9-8.3 kg (males) and 4.6-7.8 kg (females)
53.2.6	Number of animals per group	4 dogs/sex/group
53.2.7	Control animals	Yes
53.3	**Administration/ Exposure**	Oral (dietary)
53.3.1	Duration of treatment	97 days
53.3.2	Frequency of exposure	Daily
53.3.3	Postexposure period	None
53.3.4	**Oral**	
53.3.4.1	Type	In food
53.3.4.2	Concentration	In food: 0, 50, 350, and 2500 ppm equivalent to 0, 1.9, 14, 93 mg/kg bw Food consumption per day: 300 g (offered)
53.3.4.3	Vehicle	Test compound mixed with peanut oil (for dust control) then food powder
53.3.4.4	Concentration in vehicle	95%
53.3.4.5	Total volume applied	Not applicable
53.3.4.6	Controls	Diet
53.4	**Examinations**	
53.4.1	Observations	
53.4.1.1	Clinical signs	Yes, observed twice daily.
53.4.1.2	Mortality	Yes, observed twice daily.
53.4.2	Body weight	Yes, weekly.
53.4.3	Food consumption	Yes, recorded daily.
53.4.4	Water consumption	Yes, calculated weekly.
53.4.5	Ophthalmoscopic examination	Yes, at study start and in 6th week and 13th week, animals were examined for abnormalities of the eyes before and after pupil dilation using the HeineBifocal Ophthalmoscope (miroflex type).

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Repeated dose toxicity

3-month oral dog study

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53.4.6	Haematology	Yes, before the test and after 3, 6 and 13 weeks. Blood samples were collected between the hours of 7.00 and 9.30 a.m. to reduce biological variation caused by circadian rhythms. Blood samples were drawn from the jugular vein into evacuated blood collection tubes. Parameters: Haematocrit, haemoglobin, mean cell haemoglobin concentration, mean haemoglobin content of erythrocytes, erythrocyte count, total and differential leukocyte count, erythrocyte morphology, mean corpuscular volume of erythrocytes, platelet count, reticulocyte count, nucleated erythrocytes, Heinz bodies, methaemoglobin, and thromboplastin time and partial thromboplastin time.
53.4.7	Clinical Chemistry	Yes, before the test and after 3, 6 and 13 weeks. Parameters: glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, sodium, potassium, chloride, calcium, phosphorus, triglycerides, lipids, lactate dehydrogenase, creatine kinase, gamma-glutamyl transferase, ornithine carbamyl transferase, triiodothyronine (T3), thyroxine (T4), albumin, alpha-, beta- and gamma-globulins.
53.4.8	Urinalysis	Yes, before the test and after 3, 6 and 13 weeks. Parameters: colour, appearance, pH, specific gravity, protein, glucose, blood, ketone bodies, bilirubin, urobilinogen, and after sedimentation: epithelia, erythrocytes, leukocytes, casts, mucous threads and crystals
53.5	**Sacrifice and pathology**	
53.5.1	Organ Weights	Yes, at end of 13 weeks all surviving animals were sacrificed and the following organs were weighed: liver, kidneys, adrenals, testes, spleen, brain, heart, ovaries, pancreas, prostate gland, thyroid gland (with parathyroid) and lung.
53.5.2	Gross and histopathology	Yes, at end of 13 weeks all surviving animals were sacrificed and organs examined. The following organs were fixed in 4% formaldehyde solution and embedded in paraffin wax, stained with haematoxylin and eosin and examined histologically: adrenal glands, aorta, bone (sternum, femur, including articular surface), bone marrow (femur, sternum), brain, cervix, epididymides, oesophagus, eyes (fixed in Heidenhain's Susa solution), gall bladder, heart, kidneys, lachrymal gland (from nictating membrane), large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mesenteric, retropharyngeal), mammary gland area, optic nerves, prostate gland, salivary glands (mandibular, sublingual), sciatic nerve, skeletal muscle, skin, small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and all gross lesions.
53.5.3	Other examinations	Enzyme induction: At sacrifice (13 weeks) samples of liver from all animals were examined for enzyme induction, specifically: N-demethylase, O-demethylase, and cytochrome P450. Hearing impairment: Animals were tested for hearing impairment using a simple noise test before the test and at 13 weeks.

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53.5.4	Statistics	Arithmetic group means were calculated for continuous data and medians were calculated for discrete data. Body weights, organ weights and clinical laboratory data were assessed for significant differences using univariate one-way analysis of variance. If distribution was normal Dunnett test was applied to comparison of controls and treated groups, if distribution was not normal, Steel test was applied. Differences were considered significant at the 5% and 1% probability levels.
53.6	**Further remarks**	
		54 RESULTS AND DISCUSSION
54.1	**Observations**	
54.1.1	Clinical signs	No effects
54.1.2	Mortality	No treatment related mortalities were seen. One female in the control group became moribund due to severe bronchopneumonia and was sacrificed.
54.2	**Body weight**	Terminal body weights were equivalent across all groups in both males and females. Body weight gain was slightly reduced in females in the 2500 ppm group and to a lesser extent in the 350 ppm group. Body weight gain was also reduced in males in the 350 ppm group, but not the 2500 ppm group. However, due to the low magnitude and lack of dose response, this effect was not considered of any toxicological significance.
54.3	**Food consumption and compound intake**	No treatment related effects were seen on food consumption or compound intake.
54.4	**Ophthalmoscopic examination**	No effects
54.5	**Blood analysis**	
54.5.1	Haematology	There were no treatment related effects.
54.5.2	Clinical chemistry	Plasma lipid, cholesterol and triglyceride concentrations were slightly but significantly increased in both sexes in the 2500 ppm group at 3, 6 and 13 weeks. Calcium levels were slightly but significantly increased in females in the 2500 ppm dose group at 13 weeks. T3 and T4 levels were decreased in almost all treated females at 3 and 13 weeks. However these changes were not dose-and time-related, and were thus associated with liver induction rather than considered to be toxicologically relevant . Alpha -2 globulin levels were increased in females in the 2500 ppm group at 6 and 13 weeks.
54.5.3	Urinalysis	No treatment related effects were seen.
54.6	**Sacrifice and pathology**	
54.6.1	Organ weights	Liver weights were increased in both sexes in the 2500 ppm group. Thyroid weight was increased in females in the 2500 ppm group.

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54.6.2	Gross and histopathology	No treatment related macroscopic findings were noted. Centrilobular hypertrophy (liver) was seen in all animals in the high dose group. Minimal single-cell necrosis was noted in the liver in one female in the high dose group. No other treatment related lesions were noted after histopathology.
54.7	**Other**	Enzyme induction: Increased levels of N- demethylase were seen in animals of both sexes in the 2500 ppm group, although it was only statistically significant for males. Increased levels of P450 were seen in animals of both sexes in the 2500 ppm group, although it was only statistically significant for females. Hearing impairment: No treatment related effects were seen.
		55 APPLICANT'S SUMMARY AND CONCLUSION
55.1	**Materials and methods**	Groups of 4 male and female beagle dogs were given NAK 4455 for three months in diet at doses of 0, 50, 350 and 2500 ppm. All dogs were sacrificed at the end of treatment. Haematology, clinical chemistry, urinalysis, liver enzyme induction, hearing impairment tests, and gross and histopathology were performed on all animals. This study fulfils requirements of US EPA FIFRA § 82-1 (1984).
55.2	**Results and discussion**	No treatment induced changes in behaviour, appearance, mortality, food or compound intake was observed. No treatment related damage to the eye or hearing was observed. No treatment related results were seen in haematology or urinalysis studies. The results from the clinical chemistry studies and histopathology suggest that liver effects occurred in both sexes exposed to 2500 ppm. In the higher dose groups, liver weights were increased, liver enzymes were induced and centrilobular hypertrophy was observed, although no lipid vacuolation was seen, suggesting an adaptive response rather than any kind of damage. Additionally, lipids, cholesterol and triglyceride levels were all increased. Increased thyroid weights and decreased levels of thyroid hormones were seen in female animals. This may be a secondary result of altered liver physiology. The lowest adverse effect level in this study is 2500 ppm (equivalent to approximately 93 mg/kg bw) based on liver effects in both sexes. The no observable adverse effect level is 350 ppm (equivalent to approximately 14 mg/kg bw). Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is necessary
55.3	**Conclusion**	
55.3.1	LO(A)EL	The lowest adverse effect level in this study is 2500 ppm (93 mg/kg bw/day) based on liver effects in both sexes

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55.3.2	NO(A)EL	The no observable adverse effect level is 350 ppm (14 mg/kg bw/day).
55.3.3	Other	
55.3.4	Reliability	1
55.3.5	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15-03-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	The version of the applicant is adopted
Conclusion	The version of the applicant is adopted LO(A)EL: 2500 ppm both sexes (equivalent to 93 mg/kg bw/d) NO(A)EL: 350 ppm both sexes (equivalent to 14 mg/kg bw/d) 22-3-2011: The NOAEL is not 50 ppm, because there are no statistical significant effects and no dose effect relation with the dose of 350 ppm.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.4.1 (02)- 1. Main findings observed in male and female dogs after a subchronic exposure to transfluthrin

Parameter	0 ppm		50 ppm		350 ppm		2500 ppm		dose-response +/-	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	4	4	4	4	4	4	4	4		
Mortality	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4		
Clinical signs	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4		
Terminal body weight (g)	9066	8138	10240	7411	8646	7674	9027	8252	-	-
Body weight gain (g)	957	1448	1819	1536	492	1161	1139	812	-	-
Clinical chemistry										
Plasma lipids, g/L	3.4	3.1	4.1	3.4	4.4	3.5	7.2*	6.6*	-	-
Cholesterol, mM	3.95	3.79	4.83	3.98	4.76	4.10	6.81*	6.70*	-	-
Triglycerides, mM	0.45	0.43	0.53	0.47	0.62	0.49	0.95*	0.95*	-	-
Triiodothyronine (T3), nM	0.98	1.43	1.00	1.09	0.83	1.09	0.89	0.86*	-	-
Thyroxine (T4), nM	37.7	52.3	38.2	36.8*	33.0	48.7	35.6	29.2*	-	-
α-2-globulin, g/L	4.3	3.5	4.0	3.5	4.2	3.6	5.1	4.8*	-	-
P450, nmol/g	15.0	13.6	14.8	14.8	15.4	15.4	17.2	18.2*	-	-
N-demethylase, nmol/min/g	140.3	137.8	145.2	160.0	185.8*	134.1	183.4*	201.8	-	-
Liver										
Absolute weight, g	306.1	230.0	300.0	245.7	306.0	263.5	398.1**	358.7**	-	-
Centrilobular hypertrophy	0/4	0/4	0/4	0/4	0/4	0/4	4/4	4/4	-	-

Results shown only for week 13, * = $p < 0.05$; ** = $p < 0.01$

eDoc. IIIA **SECTION A6.4.2** **BPD Data set IIA/ Annex Point VI.6.4**	**Sub-chronic dermal toxicity test**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	**A 28 day repeat dose study is available, showing no route specific concerns. Effects in the 28 day study were localised to the treatment site.** **Route to route extrapolation is possible, therefore a specific sub-chronic dermal study has not been conducted as this is not an appropriate use of animals.**	
Undertaking of intended data submission [ ]		
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	15-03-2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable	
Conclusion	The justification of the applicant is adopted	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE (specify)	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

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	56 REFERENCE	
56.1	**Reference**	[redacted] (1989). NAK 4455. Study for subchronic inhalation toxicity to the rat. [redacted] [redacted] Report No. T 4029634 [BES Ref: MO-03-009231] Report date: October 10, 1989 Unpublished
56.2	**Data protection**	Yes
56.2.1	Data owner	Bayer CropScience
56.2.2	Companies with letters of access	
56.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	57 GUIDELINES AND QUALITY ASSURANCE	
57.1	**Guideline study**	Yes OECD Guideline 413 Subchronic Inhalation Toxicity (1981) US EPA FIFRA § 82-4 Subchronic Inhalation Toxicity (1984)
57.2	**GLP**	Yes
57.3	**Deviations**	No
	58 MATERIALS AND METHODS	
58.1	**Test material**	NAK 4455 (transfluthrin)
58.1.1	Lot/Batch number	250987
58.1.2	Specification	As given in section 2
58.1.2.1	Description	Grey brown/solid crystalline mass (room temperature)
58.1.2.2	Purity	95.0%
58.1.2.3	Stability	Test substance was stored at room temperature in laboratory cabinet and kept stable throughout the study. Test compound was melted overnight at 40ºC in a drying cabinet before stock solution was mixed. Regular checks confirmed integrity of melted compound.
58.2	**Test Animals**	
58.2.1	Species	Rat

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58.2.2	Strain	Bor:WISW (SPF-Cpb) (Wistar)
58.2.3	Source	[redacted]
58.2.4	Sex	Male and female
58.2.5	Age/weight at study initiation	2-3 months Mean body weight at start of study: 190 g (males) and 175 g (females)
58.2.6	Number of animals per group	10 rats/sex/group (except vehicle control and 1000 mg/m^3 groups which had an additional 10 animals/sex/group—"satellite groups")
58.2.7	Control animals	Yes, air control and vehicle control animals
58.3	**Administration/ Exposure**	Inhalation
58.3.1	Duration of treatment	13 weeks
58.3.2	Frequency of exposure	5 days per week
58.3.3	Postexposure period	4 weeks for satellite groups
58.3.4	**Inhalation**	
58.3.4.1	Concentrations	Nominal: 0, 40, 250, 1000 [mg/m³] Analytical: 0, 4.9, 46.7, 220.2 [mg/m³]
58.3.4.2	Particle size	MMAD 1.1 [µm] ± GSD 1.4 [µm]
58.3.4.3	Type or preparation of particles	The aerosol was sprayed under dynamic conditions, using a nozzle and compressed air into a cylindrical inhalation chamber with baffle chamber. The conditions of generation of the aerosol ensure about 30 air exchanges per hour. Air flow was monitored continuously. The air samples for analytical determination and particle distribution were taken in the rats' immediate inhalation area. NAK 4455 concentration was analysed by GC. Particle distribution was analysed with an aerodynamic particle size with Laser Velocimeter.
58.3.4.4	Type of exposure	Nose/head only
58.3.4.5	Vehicle	Polyethylene glycol E 400/ethanol (1:1)
58.3.4.6	Concentration in vehicle	0.4, .5, and 10 % (w/v)
58.3.4.7	Duration of exposure	6 h
58.3.4.8	Controls	Sham exposed and vehicle exposed
58.4	**Examinations**	
58.4.1	Observations	

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58.4.1.1	Clinical signs	Yes, observed at least twice daily—in particular for: Appearance of visible mucous of eyes and respiratory tract; general state of muzzle skin and ear scoops, state of coat, grooming activities, respiration, circulation, somatomotor system and behaviour pattern (including tremor, convulsions, hypersalivation, dyspnoea, diarrhoea, lethargy, sedation and coma), central nervous and autonomic symptoms. Additionally, in the 4th, 8th and 12th week the following reflexes were tested: cornea, pinna, myotactic, light, startle, and righting
58.4.1.2	Mortality	Yes, observed twice daily.
58.4.2	Body weight	Yes, before study, then weekly.
58.4.3	Food consumption	Yes, calculated weekly.
58.4.4	Water consumption	No
58.4.5	Ophthalmoscopic examination	Yes, before start of study and week 13, 5 animals/group/sex were examined for abnormalities of the eyes after pupil dilation, specifically alterations of the retina, vitreous body, lens, cornea, and external surface of the eye.
58.4.6	Haematology	Yes, for all animals at end of study (13 or 17 weeks). The blood samples were taken by heart puncture from the anaesthetised (diethyl ether) rats. Several parameters (marked with a * below) were also measured at 4 and 8 weeks in 5 animals/sex/group (For the interim examinations the blood samples were obtained by puncture of the retrobulbar venous plexus by means of a heparinised glass capillary). Parameters: Haematocrit*, haemoglobin*, leukocytes*, erythrocytes*, mean cell haemoglobin concentration*, mean haemoglobin content of erythrocytes*, differential blood count, reticulocytes, thrombocytes, mean corpuscular volume of erythrocytes* and coagulation time*.
58.4.7	Clinical Chemistry	Yes, for all animals at end of study (13 or 17 weeks). Several parameters (marked with a * below) were also measured at 4 and 8 weeks in 5 animals/sex/group. Parameters: glucose, total cholesterol, urea, total bilirubin, creatinine, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glutamate dehydrogenase, phosphate*, magnesium*, sodium, potassium, chloride, calcium, triglycerides, albumin, lactate, lactate dehydrogenase, creatinine kinase, protein electrophoresis (albumin, α1, α2, β and γ globulins), fluoride (in bones and teeth).
58.4.8	Urinalysis	Yes, at end of study following ca. 8 – 16 hours fast. Parameters: volume, pH, specific gravity, sodium, potassium, phosphate, protein, glucose, blood, ketone bodies, bilirubin, urobilinogen, and after sedimentation: bacteria, epithelia, erythrocytes, leukocytes, protein casts and crystals.
58.5	**Sacrifice and**	

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	pathology	
58.5.1	Organ Weights	Yes, at end of treatment/observation period all surviving animals were sacrificed and the following organs were weighed: liver, kidneys, adrenals, testes, spleen, brain, heart, ovaries, thyroid gland, thymus and lung.
58.5.2	Gross and histopathology	Yes, at end of 13 or 17 weeks all surviving animals were sacrificed under diethyl ether anaesthesia by exsanguination (heart puncture) and organs examined. The following organs were fixed in 10% formaldehyde solution and embedded in paraplast and stained with haemalum eosin: adrenal glands, aorta, bone (femur, sternum), bone marrow (femur, sternum), bone marrow morphology (smear), brain, cervix, coagulation gland, epididymides, oesophagus, eyes, Harderian gland, head (nasopharynx, oropharynx, sinus nasals and paranasales), heart, hypophysis, kidneys with pelvis, lachrymal gland, large intestine (cecum, colon, rectum), larynx, liver, lungs (instillation fixation), lymph nodes (mesenteric, cervical/mandibular, mediastinal), mammary gland, optic nerves (not examined), ovaries, pancreas, parathyroid, prostate, salivary glands, sciatic nerve, seminal vesicles with seminal duct, skeletal muscle, skin (muzzle and mammary areas), small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach, testes, thymus, thyroid gland, tongue, trachea, urinary bladder (instillation fixation), uterus, and vagina, vas deferens.
58.5.3	Other examinations	Enzyme induction: At sacrifice (13 or 17 weeks) a sample of liver from all animals was examined for triglyceride levels and enzyme induction, specifically: N-demethylase, O-demethylase, and cytochrome P450. Pulmonary function: Toward end of exposure/observation, pulmonary function was examined in 5 rats/sex/group, specifically: respiration rate, respiration minute volume, pleural pressure, dynamic compliance, static compliance specific compliance, pulmonary resistance, forced expiratory measurements (peak expiratory volume, mean mid-expiratory flow, FEV, FEF), tidal volume, inspiration capacity, expiration capacity, vital capacity, total lung capacity, residual volume, functional residual capacity, CO diffusion capacity, acetylcholine provocation test
58.5.4	Statistics	For body weights, organ weights and clinical chemical data, arithmetic means and standard deviations were calculated and statistically evaluated using Mann and Whitney's rank test with Walter's modification at significance levels of 0.05 and 0.01. Additionally, organ weights, pulmonary function test results, bone marrow morphology and fluoride concentrations were statistically evaluated using one way analysis of variance at a significance level of 0.05. The histopathology results were evaluated using a "pairwise Fisher's test."
58.6	**Further remarks**	

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18-Week inhalation rat study

59 RESULTS AND DISCUSSION

59.1	**Observations**	
59.1.1	Clinical signs	Hyperactivity after exposure (resolving by the following day) was seen in all animals in the 1000 mg/m^3 group throughout the entire exposure period. In the first week, animals in the high dose group also demonstrated bristling and ungroomed coats and tremor after exposure, resolving by the following day. These signs gradually declined after the 2nd week of exposure. Some animals in the high dose group had bloody noses. No effects were seen in the 40 and 250 mg/m^3 groups.
59.1.2	Mortality	The original high dose in the study was (nominal) 1500 mg/m^3. At that dose, 4 female rats died during the first exposure. These rats were replaced with others and, from the 2nd exposure in the study, the high dose was 1000 mg/m^3. Aside from the 4 rats dying at 1500 mg/m^3, which are not included in the study statistics, there were no treatment related mortalities. One female animal in the high group died in the 12th week due to suffocation in the exposure tube.
59.2	**Body weight gain**	No treatment related effects were seen.
59.3	**Food consumption and compound intake**	No treatment related effects were seen on food consumption or compound intake.
59.4	**Ophthalmoscopic examination**	No effects
59.5	**Blood analysis**	
59.5.1	Haematology	Females in the high dose group had statistically significant increased percentages of polymorphonuclear neutrophils. No other treatment related effects were seen.
59.5.2	Clinical chemistry	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and were within normal physiologic parameters for the effect. Males in the high dose group (regular and satellite) had statistically significant increased levels of fluoride in the bones. Females in the high dose group (regular only) had statistically significant increased levels of fluoride in the teeth. In the satellite groups, alanine aminotransferase levels were increased in males, as was urea. In females sodium levels were reduced and potassium levels increased.
59.5.3	Urinalysis	No treatment related effects were seen in male animals. In females, pH was decreased in vehicle and all treated groups, and phosphate was increased in all treated groups. Volume was decreased and density increased in females in the high dose group. The changes were not dose-related and/or within the range of normal values, they were thus not considered to be of any toxicological relevance.

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59.6	**Sacrifice and pathology**	
59.6.1	Organ weights	No treatment related effects were seen.
59.6.2	Gross and histopathology	No treatment related macroscopic findings were noted. The bone marrow smear revealed that polymorphic neutrophils were significantly reduced in the mid and high group males although this had resolved by the sacrifice of the satellite group. In females, polymorphic neutrophils were nonsignificantly reduced in the high group at 13 weeks and significantly reduced at 17 weeks. There were a number of other effects in bone marrow cells, all of which appear to be reversible (i.e., not found in the satellite group) including, reduced monocytes in mid and high group males and females, increased normoblasts in all male treatment groups, decreased plasma cell in males in the vehicle control and all treatment groups. No other treatment related microscopic lesions were found after the histopathology examination.
59.7	**Other**	Enzyme induction: No treatment related effects were seen. Pulmonary function: No treatment-related effects were seen.

60 APPLICANT'S SUMMARY AND CONCLUSION

60.1	**Materials and methods**	In a 18-week inhalation study, groups of 10 male and female Wistar rats were head/nose exposed to NAK 4455 for 13 weeks in air at doses (analytical concentrations) of 0 (air control and vehicle control), 4.9, 46.7, and 220.2 mg/m^3. The MMAD was 1.1 µm, the geometric standard deviation was approximately 1.4 µm, making the particles readily inhalable. An additional 10 animals/sex/group were exposed to vehicle alone or 220.2 mg/m^3 NAK4455 for 13 weeks and then observed for 4 weeks without exposure (satellite groups). All rats were sacrificed at the end of treatment or end of observation period. Haematology, clinical chemistry, urinalysis, liver enzyme induction, fluoride level measurements, pulmonary function tests, ophthalmological examinations and gross and histopathology were performed on all animals. This study exceeds requirements of OECD 413 Subchronic inhalation study (1981) and US EPA FIFRA § 82-4 (1984).
60.2	**Results and discussion**	The major finding in this study was post-exposure hyperactivity (resolving by the following day) in all animals in the 1000 mg/m^3 group throughout the entire exposure period. In the first week, animals in the high dose group also demonstrated bristling and ungroomed coats and tremor after exposure, resolving by the following day. These signs gradually declined after the 2nd week of exposure. No treatment induced changes in mortality, food or compound intake was observed. No treatment related damage to the eye was observed. No treatment related results were seen on pulmonary function or enzyme induction. Fluoride levels in bone (males) and teeth (females) were increased in the

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high dose group.

High dose group females had increased polymorphonuclear neutrophils (PMN) in the blood and decreased PMN in the bone marrow. As there does not appear to be a change in absolute white blood cell numbers, this is not judged to be an effect of concern.

The combined results of the haematology, clinical chemistry and urinalysis evidenced minor effects which might indicate slight liver and kidney effects. However, these results are neither time nor dose-dependent and are not supported by the histopathological results. Additionally, the effects, while statistically significant, remain largely within normal physiological parameters. Thus, they are concluded not to be of toxicological relevance.

There was no effect on organ weight, no macroscopic changes were seen in the organs, and with the exception of a slight hyperaemia in some organs—likely due to sacrifice—there were no microscopic changes of note.

If a respiratory minute volume of 1 L/kg rat is assumed, then the approximate mg/kg bw-day equivalencies for the exposure doses are:

Nominal (mg/m^3)	Analytical (mg/m^3)	Analytical (mg/L)	Nominal (mg/kg bw-day)
0 (air)	0 (air)	0 (air)	0 (air)
0 (vehicle)	0 (vehicle)	0 (vehicle)	0 (vehicle)
40	4.9	0.005	1.8
250	46.7	0.047	16.8
1000	220.2	0.220	79.3

The lowest adverse effect level in this study is 220.2 mg/m^3 based on neurological effects in both sexes. The no observable adverse effect level is 46.7 mg/m^3.

60.3 Conclusion

60.3.1 LO(A)EL — The lowest adverse effect level in this study is 220.2 mg/m^3 (approximately 79 mg/kg bw/day) based on neurological effects (increased post-exposure activity and tremor) in both sexes.

60.3.2 NO(A)EL — The no observable adverse effect level is 46.7 mg/m^3, approximately equivalent to 16.8 mg/kg bw/day.

60.3.3 Other

60.3.4 Reliability — 1

60.3.5 Deficiencies — No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	16-03-2007
Materials and Methods	The version of the applicant is acceptable
Results and discussion	The version of the applicant is adopted
Conclusion	LO(A)EL: 220.2 mg/m^3 (approximately equivalent to 79 mg/kg bw/d) NO(A)EL: 46.7 mg/m^3 (approximately equivalent to 17 mg/kg bw/d) *22-3-2011: N*o changes in absolute numbers, largely within normal physiological parameters, no macro/microscopic changes in organs are found. Therefore 4,9 mg/m3 is not an appropriate NOEC for the derivation of an AELacute (also no risk is expected based on this value). There is no evident dose response relation seen in the bone marrow smears.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_4.3-1. Main findings observed in male and female rats following a subchronic inhalation exposure to transfluthrin

Parameter	**Control (air)**		**Control (vehicle)[a]**		**4.9 mg/m³**		**46.7 mg/m³**		**220.2 mg/m³ [a]**		**dose-response +/-**	
	m	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**	**m**	**f**
Number of animals examined	10	10	20	20	10	10	10	10	20	20		
Mortality[b]	0/10	0/10	0/20	0/20	0/10	0/10	0/10	0/10	0/20	1/20		
Clinical signs	0/10	0/10	0/20	0/20	0/10	0/10	0/10	0/10	20/20	20/20		
Body weight	0/10	0/10	0/20	0/20	0/10	0/10	0/10	0/10	0/20	0/20		
Food consumption	0/10	0/10	0/20	0/20	0/10	0/10	0/10	0/10	0/20	0/20		
Clinical chemistry												
Glutamate dehydrogenase, u/L	0.9	1.7	2.0 (1.5)	4.1 (1.7)	1.5	0.9	0.3	1.7	3.2** (0.8)	1.0 (0.3)		
Triglycerides, mM	0.49	0.8	0.56 (0.63)	0.55* (0.64)	0.46	0.49**	0.55	0.62*	0.48 (0.91)	0.88 (1.01)	-	-
Creatinine, µM	58	53	51 (54)	56 (53)	65	47**	49**	46**	56 (51)	50 (51)	-	-
Urea, mM	8.11	8.08	6.50** (6.63)	7.01* (7.30*)	6.71**	6.07**	6.81**	6.52**	7.66 (6.25)	7.65 (6.35)	-	-
Glucose, mM	4.14	3.61	4.05 (4.09)	3.74 (4.00)	3.82	4.04**	3.95	3.96*	4.35 (4.43)	4.17** (4.53)	-	-
Calcium, mM	2.56	2.57	2.58 (2.61)	2.55 (2.64)	2.59	2.54	2.58	2.52*	2.59 (2.62)	2.46** (2.63)	-	-
Sodium, mM	145	143	144 (146)	144 (145)	144	144	144	144	145 (145)	142* (144**)	-	-
Fluoride (bone), mg/g ash	0.35	0.49	0.35 (0.32)	0.51 (0.53)	0.37	0.55	0.40	0.54	0.46** (0.42*)	0.58 (0.55)		
Fluoride (teeth), mg/g ash	0.14	0.14	0.11 (0.11)	0.13 (0.14)	0.11	0.14	0.12	0.14	0.16 (0.13)	0.21* (0.15)		
Haematology												
Polymorphonuclear neutrophils, %	10.1	10.2	11.2 (12.3)	10.1 (7.5)	10.4	9.5	12.4	9.7	9.3 (14.5)	13.9* (5.7)		
Urinalysis	0/4			0/4	0/4	0/4	0/4	0/4	0/4	0/4		
pH	7.19	7.27	7.09 (8.23)	6.69* (7.4)	7.40	6.74*	7.35	6.57**	6.90 (8.04)	6.34** (7.0)		
Phosphorus, mM	38.1	26.5	42.5 (19.5)	35.7 (33.7)	38.7	33.8*	34.8	35.9*	45.8 (24.4)	59.3** (32.5)		
Volume, mL	6	5	7 (17)	6 (8)	8*	5	8	6	7 (12)	4* (8)		
Density, g/L	1023	1018	1022 (1014)	1019 (1022)	1020	1018	1021	1019	1025 (1018)	1023** (1024)		
Bone marrow smear (all per 1000 counted cells)												
PMN	8	5	9 (10)	5 (2**)	9	5	3**	3	2** (7)	3 (2**)		

Normoblasts	14	27	19 (22)	92** (27)	26**	31	54**	39	51** (34)	43 (38)		
Lymphocytes	129	95	116 (145)	84 (110)	94	81	42**	99	88 (116)	75 (141)		
Monocytes	10	5	9 (10)	4 (3)	7	3	1**	1**	2** (5)	2** (3)		
Plasma cells	8	7	5* (7)	5 (6)	5*	6	3**	5	4**(5)	5 (4)		

[a]For vehicle control and high dose satellite groups, response, where relevant, is in parentheses.

[b]The original nominal high dose in the study was 1500 mg/m^3. When this exposure concentration was used, 4 female animals died on the first day. These deaths are not reflected on the table. The animals were replaced, and from the 2nd day, the nominal high dose exposure was 1000 mg/m^3.

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

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61 REFERENCE

61.1	**Reference**	[redacted] (1993). NAK 4455. Study for chronic toxicity and carcinogenicity in Wistar rats (Administration in diet for 2 years). [redacted] Report No. T 8025696 [BES Ref: MO-03-009856] Report date: July 7, 1993. Unpublished.
61.2	**Data protection**	Yes
61.2.1	Data owner	Bayer CropScience
61.2.2	Companies with letters of access	
61.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

62 GUIDELINES AND QUALITY ASSURANCE

62.1	**Guideline study**	Yes OECD 453 (1981) US EPA FIFRA § 83-5 (1984)
62.2	**GLP**	Yes
62.3	**Deviations**	No

63 MATERIALS AND METHODS

63.1	**Test material**	NAK 4455 (transfluthrin)
63.1.1	Lot/Batch number	Mixed batch no: 130187, from 10.11.87: 250987
63.1.2	Specification	As given in sections 2 and 3
63.1.2.1	Description	Brown-yellow clear liquid after heating to 50ºC
63.1.2.2	Purity	95.0% (130187), 94.5% (250987)
63.1.2.3	Stability	Test compound content in the administered formulation was verified at the start of study, and approximately every 3 months thereafter. Stability and homogeneity were verified before beginning of study. Purity of 100% was assumed for the technical test compound. Food mixes contained 1% peanut oil to minimize dust generation.
63.2	**Test Animals**	

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63.2.1	Species	Rat
63.2.2	Strain	Wistar; Bor:WISW (SPF-Cpb)
63.2.3	Source	[redacted]
63.2.4	Sex	Male and female
63.2.5	Age/weight at study initiation	4-6 weeks Weight range at start of study 54-78 g (males) and 52-73 g (females)
63.2.6	Number of animals per group	70 rats/sex/group
63.2.6.1	At interim sacrifice	10 animals/group/sex at 12 months
63.2.6.2	At terminal sacrifice	60 animals/group/sex
63.2.7	Control animals	Yes
63.3	**Administration/ Exposure**	Oral (dietary)
63.3.1	Duration of treatment	25 months
63.3.2	Interim sacrifice(s)	After 12 months
63.3.3	Final sacrifice	After 25 months
63.3.4	Frequency of exposure	Daily (continuous in diet)
63.3.5	Postexposure period	None
		Oral
63.3.6	Type	In food
63.3.7	Concentration	Food 0, 20, 200, 2000 ppm, equivalent to: Males: 0, 1.0, 9.9, 100.4 mg/kg bw-day Females: 0, 1.4, 13.6, 142.1 mg/kg bw-day Food consumption per day ad libitum
63.3.8	Vehicle	Moistened with peanut oil/ mixed into food (1% final concentration)
63.3.9	Concentration in vehicle	N/A
63.3.10	Total volume applied	Not applicable
63.3.11	Controls	Diet with peanut oil

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63.4	**Examinations**	
63.4.1	Body weight	Yes, before administration of first dose and then weekly.
63.4.2	Food consumption	Yes, measured weekly.
63.4.3	Water consumption	Yes, measured weekly.
63.4.4	Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
63.4.5	Macroscopic investigations	Location and progression of palpable masses, skin tumours were recorded
63.4.6	Ophthalmoscopic examination	Yes, at start of study and after 12 and 24 months for 10 male and 10 female animals in 0 and 2000 ppm groups—surroundings of the eyes and the anterior eye sections were examined for alterations, pupil reflex test was made in a darkened room, transparent eye media and eye fundus were examined after pupil dilation.
63.4.7	Haematology	Yes, blood samples were taken in the morning from the retro-orbital venous plexus (under anaesthesia). Number of animals: 10 or 20 animals/sex/group Time points: after 6, 12, 18, 24 months of treatment Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, thrombocyte count, thromboplastin time, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular cell volume (MCV), erythrocyte morphology
63.4.8	Clinical Chemistry	Yes, for determination of glucose, blood samples were taken in the morning from unfasted, unanaesthetised animals from one of the caudal veins. Blood samples for other parameters were taken in the morning from the retro-orbital venous plexus (under anaesthesia). Number of animals: 10 or 20 animals/sex/group Time points: after 6, 12, 18, 24 months of treatment Parameters: sodium, potassium, phosphate, calcium, chloride, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, triglycerides
63.4.9	Urinalysis	Yes, a few days before blood sampling after approx. 16-hr fast (water ad lib. Number of animals: 10 animals/sex/group Time points: after 6, 12, 18, 24 months of treatment Some parameters only measured at end of study, they are marked with a * below. Parameters: appearance, volume, osmolality*, specific gravity, pH, protein, glucose, blood, bilirubin, ketone bodies, urobilinogen, sediment (leukocytes, erythrocytes, epithelia, cylinders (protein casts) and others, e.g. bacteria, crystals), creatinine*, urea*, phosphate*, calcium*, potassium*, sodium*, chloride*

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63.4.10 Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at interim and final sacrifice
63.4.10.1 Organ Weights	Yes, from all animals at interim or final sacrifice Organs: liver, kidneys, adrenals, testes, spleen, brain, heart, and lungs
63.4.11 Histopathology	Yes, from all animals at interim and terminal sacrifices. Organs: fixed in 10% buffered formaldehyde solution (urinary bladder and lungs fixed by instillation of formaldehyde solution): adrenal glands, aorta, brain (cerebrum, cerebellum, brain stem), epididymides, oesophagus, eyes (including lids and optic nerves), femur, Harderian glands, "head" (nasal and oropharyngeal cavity), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum, remaining intestinal tissue), kidneys, lachrymal glands (extraorbital), larynx, liver, lungs, mandibular lymph node, mesenteric lymph node, ovaries (including oviducts), parathyroid glands, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, skin/mammary region, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, ureter, urethra, urinary bladder, uterus, vagina and any other tissue showing changes.
63.4.12 Other examinations	Enzyme induction: At sacrifice (interim and final) 5 animals/sex/group were examined for enzyme induction in the liver, specifically: N-demethylase, O-demethylase, cytochrome P450 and carnitine acyl transferase (CAT). Fluoride content: at sacrifice (interim and final), the teeth and bones of 5 animals/sex/group were analysed for fluoride levels.
63.5 Statistics	Arithmetic group means and standard deviation were calculated for all quantitative results (except fluoride data). Test collective data were compared with control collective data using either Mann and Whitney or Wilcoxon's U test. Differences were considered significant at the 5% and 1% probability level. Data from the fluoride analysis were evaluated using Dunnett's test after one-factor analysis of variance. Comparison of survival curves used Wilcoxon's generalized test (Breslow test), a weighting proportional to respective group sized per event time.
63.6 Further remarks	
	64 RESULTS AND DISCUSSION
64.1 Mortality	No treatment-related mortality was observed among all treated groups. Survival rate was equivalent across all groups.
64.2 Body weight	Male animals in the high and mid dose groups were slightly but significantly heavier than control animals intermittently throughout the study, although the effect appeared most frequent between weeks 33 and 90. Nevertheless, due to the lack of dose-relationship, these changes were considered to be devoid of any toxicological significance. Female animals in the high dose group had a slight but significant reduction in

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		weight intermittently throughout the study.	
64.3	**Food consumption**	No treatment related effects were seen.	
64.4	**Water consumption**	No treatment related effects were seen on female animals. Males in the high dose group had a slight but significantly increased water intake.	
64.5	**Clinical signs**	No treatment related effects were seen	
64.6	**Macroscopic investigations**	No treatment related effects were seen.	
64.7	**Ophthalmoscopic examination**	No treatment related effects were seen	
64.8	**Haematology**	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, haemoglobin levels tended to be minimally reduced in high dose males and females, haematocrit was reduced in high dose males, and mean cell haemoglobin was reduced in all treated males, throughout the study.	
64.9	**Clinical Chemistry**	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, triglyceride levels appear to be reduced in all treated males and in high dose females. The absence of clear dose-response or of corroborative change in other parameters, suggests that the change may in part be due to fortuitously higher values in controls.	
64.10	**Urinalysis**	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. The only consistent effect appeared to be slightly but significantly reduced density of urine in all treated males and mid and top-dose females at the 6 month time point.	
64.11	**Pathology**	Interim autopsy: No treatment related effects were found up to and including the 200 ppm dose group. Seven of ten males in the 2000 ppm dose group were found to have rough kidney surfaces. Final autopsy: Liver changes (swollen, thickened, enlarged and/or presence of nodules) were noted in a few males in each treatment group and in females in the 200 and 2000 ppm dose groups. Additionally, 2 females in the 2000 ppm dose group were found to have urinary bladder nodules.	
64.12	**Organ Weights**	Absolute and relative kidney and liver weights were increased in males and females in the high dose groups. At the 12-month interim autopsy, absolute kidney weight in females in the 200 ppm group was elevated. At the 24-month final autopsy absolute kidney weight was also increased in males and females in the 200 ppm dose group, at was relative kidney weight in males in the 200 ppm group and relative liver weight in all treated females.	X

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64.13	**Histopathology**	Interim autopsy: Glomerulonephrosis was seen in males in the 200 and 2000 ppm dose groups, yellow-brown pigment deposits were seen in the tubular epithelial cells and interstitial tissue of the kidneys of both male and female animals in the 200 and 2000 ppm dose groups in an apparently dose-dependent manner. Males in the high dose group had an increased incidence of cuboid cells in the follicular epithelium of the thyroid. Final autopsy: Glomerulonephrosis was increased in males in the 200 and 2000 ppm dose groups and in females in the 20 and 200 ppm dose groups. Pigment deposition was increased in males and females in the 200 and 2000 ppm dose groups. An increased incidence of urothelial hyperplasia of urinary bladder was seen in high dose group animals, as was a slightly increased rate of thyroid hyperplasia.
64.14	**Other examinations**	Enzyme induction: O-demethylase was higher in male and female animals in the high dose group at the 12 month but not 24 month sacrifice. Cytochrome P450 was higher in all female treatment groups at 12 but not 24 months, and in high dose males at 12 but not 24 months. Carnitine acyl transferase was higher in high dose group females at 12 and 24 months. Fluoride incorporation: Fluoride levels in bones and teeth of male and female animals were statistically significantly increased in the 200 and 2000 ppm groups at both 12 and 24 months.
64.15	**Time to tumours**	No treatment related effects were seen.
64.16	**Other**	Neoplastic lesions: No treatment related neoplastic lesions were seen at the interim autopsy. At the 24-month final autopsy a miscellany of benign and malignant tumours were seen an all groups (including controls) and were clearly not treatment related (lack of dose response, increased incidence in controls, single instance in middle dose group, etc). None of the tumours showed statistical significance for trend based on combined prevalence and death rate method of Peto. Two or 3 hepatocellular adenomas (benign) were seen in each of the male treatment groups and not in the controls or female animals. This response was within the parameters of historical incidence of this tumour. In the adrenal glands, there was an increased incidence of medullary tumours (benign) in the male treatment groups. In the female high dose group, there was an increased incidence in mammary adenoma (benign), two lipomatous tumours (1 malignant and 1 benign) were observed in the kidneys of high dose group males, Both sexes exhibited an increased incidence of hyperplasia and also tumours (1 or 2 papilloma and carcinoma) of the urinary bladder after the administration 2000 ppm of the test substance. The tumour frequency was above historical control data.

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65 APPLICANT'S SUMMARY AND CONCLUSION

65.1 Materials and methods

Groups of 70 male and female Bor: WISW (SPF-Cpb) rats were given NAK 4455 in the diet at concentrations of 0, 20, 200 and 2000 ppm for 12 months at which point 10 rats/sex/group were sacrificed (interim autopsy). The remaining 60 rats/sex/group were given NAK 4455 in the diet for an additional 12 months before sacrifice. Haematology, clinical chemistry, urinalysis, liver enzyme induction, measurement of fluoride levels, and gross and histopathology were performed on all animals at or just before sacrifice. Additionally, haematology, clinical chemistry and urinalysis were performed at 6, 12, 18 and 24 months. This study fulfils the requirements of OECD 453 (1981) and US EPA FIFRA § 83-5 (1984).

65.2 Results and discussion

No treatment induced changes in behaviour, appearance, mortality, food or compound intake was observed. No treatment related damage to the eye was observed.

The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occur in both sexes exposed to 2000 ppm and likely begins at 200 ppm.

In the higher dose groups, liver weights were increased, liver enzymes were induced and enlarged liver was observed. Additionally, triglyceride levels were decreased. In the treated male groups, benign hepatocellular adenomas were seen. These were within the limits of historical controls and were not statistically significant for trend.

Also in the higher dose groups, increased kidney weights, decreased urine density (at 6 months), increased water consumption (males only) were observed. Rough kidney surfaces were noted in high dose group males, and glomerulonephrosis and pigment deposits within the kidneys were seen in 200 and 2000 ppm dose groups. Two lipomatous tumours were observed in the kidneys of high dose group males, but these are not statistically significant and do not demonstrate a dose-response. Increased incidence of urothelial hyperplasia of urinary bladder was seen in high dose group animals. A slight, non-significant increase of (urothelial) tumours was seen in the urinary bladder of high-dose animals. It seems likely that both kidney and urinary bladder tumours are secondary to cell damage and cell proliferation.

A dose dependent increase in fluoride content in teeth and bones was seen starting at 20 ppm; the increase became statistically significant at 200 ppm.

Incidence of thyroid hyperplasia was slightly increased in high dose group animals. This may be a secondary result of altered liver physiology.

The lowest adverse effect level in this study is 200 ppm (equivalent to approximately 9.9 and 13.6 mg/kg bw-day for males and females respectively) based on liver and kidney damage in both sexes. The no observable adverse effect level is 20 ppm (equivalent to approximately

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		1.0 and 1.4 mg/kg bw-day for males and females respectively).	
65.3	**Conclusion**		X
65.3.1	Reliability	1	
65.3.2	Deficiencies	During a single spot-check analysis of the homogeneity of the compound in the feed (approximately 12 weeks after start of study), it was found that the container labelled 2000 ppm contained 200 ppm feed and vice versa. The researchers were unable to determine if the containers had simply been mislabelled or if the rats had been feed the incorrect dose for their group. Even if the rats had been feed the incorrect dose, a single instance over 104 weeks should not have any significant effect on the cumulative dose received, nor should it have had an effect on blood parameters, as the first blood sample was taken 6 months after beginning of study. This deficiency is not expected to have any effect on the study.	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22 January 2007
Materials and Methods	The description of the applicant is acceptable
Results and discussion	Organ weight. The applicant states that at 24 months relative liver weight was increased in all female groups. However, relative liver weight actually was significantly decreased (up to 5%) in females at 20 and 200 ppm.
Conclusion	The applicant does not draw conclusions. Transfluthrin induces glomerulonephrosis at 200 ppm and higher. The urinary bladder urothelial hyperplasia, thyroid follicular hyperplasia and increased cuboidal cells (m+f) and urinary bladder tumours (papilloma and carcinoma), observed at 2000 ppm, are considered to be treatment-related. The tumours in thyroid and liver are considered not related to treatment. Based on the effects observed in the kidney (glomerulonephrosis, pigment deposition, increased absolute and relative weight) the LOAEL is 200 ppm, equal to 9.9 mg/kg bw/day. The NOAEL is 20 ppm, equal to 1.0 mg/kg bw/day. 22-03-2011: Based on new data the conclusion on carcinogenicity is adjusted, see discussion in Doc IIA. In the appendix written by the applicant the kidney effects are summarized (see also 6.8.2).
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.5(01) -1. Main haematological and clinical chemistry findings observed in male and female rats during long term exposure to transfluthrin

Affected		0 ppm				20 ppm				200 ppm				2000 ppm			
Parameter, unit, sex		Months after start of treatment															
Haematology		6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
Hb, g/L	M	160	161	161	160	153	157	158	158	154	156	158	154	154	152**	153**	153
	F	153	151	150	154	149**	149	148	152	146*	147	149	145**	146**	147	144*	147
HCT, L/L	M	0.482	0.493	0.507	0.501	0.470	0.479	0.503	0.493	0.475	0.479	0.503	0.487	0.474	0.472*	0.485**	0.487
	F	0.458	0.461	0.467	0.476	0.447	0.455	0.467	0.477	0.444	0.451	0.467	0.457	0.437**	0.450	0.458	0.461
MCH, pg	M	18.5	17.5	18.0	18.2	17.7**	16.9*	17.1*	17.3	17.6**	16.8*	17.1*	17.2*	17.5*	16.7	17.2*	17.1*
	F	19.0	17.9	18.4	18.8	18.7	17.8	19.0	18.3	18.3	17.5	18.0	18.2	18.8	17.8	18.3	18.4
Thrombocytes, 10^9/L	M	1018	1092	1001	971	979	1057	994	999	1001	1036	989	1042	890	1002	900	934
	F	889	999	862	791	826	970	886	872	911	961	846	827	944	1054	925	910*
Clinical Chemistry																	
ASAT, U/L	M	32.8	29.9	35.5	32.1	37.4	35.6*	36.7	35.9	34.1	31.6	33.5	40.8	38.6	30.9	36.5	34.0
	F	34.0	71.9	82.3	61.2	40.2	41.9	61.2	54.4	31.2*	40.0	89.1	77.6	30.9**	33.8*	93.8	75.5
Triglycerides, mM	M	2.10	3.04	2.78	2.84	1.25**	1.69*	2.08*	2.27	1.24**	1.61*	2.38	2.32	1.08**	0.93**	1.49**	1.66**
	F	1.63	1.75	1.39	1.75	1.15*	1.64	1.50	1.72	1.28	1.40	1.46	1.65	0.82**	0.91**	1.02*	1.2
Months		12		24		12		24		12		24		12		24	
N-Dem, mU/g	M	143.0		83.0		125.5		83.0		114.8		80.5		175.4		89.7	
	F	81.8		70.7		73.2		70.6		66.3*		56.7*		90.0		62.0	
O-Dem, U/g	M	12.5		13.0		11.8		11.3		12.4		11.0		17.6**		13.2	
	F	11.6		10.1		12.5		11.1		13.0		10.6		16.5**		11.6	
P450, nmol/g	M	32.0		38.3		30.6		30.9**		30.3		35.2		49.6**		30.0*	
	F	29.8		34.6		39.2*		38.6		35.6*		42.6		52.9**		38.5	
CAT, U/g	M	0.52		0.56		0.39**		0.55		0.45		0.46		0.61		0.83	
	F	1.24		1.86		1.21		2.03		1.56		1.92		2.86**		2.65*	
Fluoride, mg/g ash bones	M	0.459		0.662		0.514		0.777		0.756*		1.337*		2.243*		2.814*	
	F	0.548		0.894		0.637		0.848		1.249*		1.485*		2.949*		2.793*	
Fluoride, mg/g ash teeth	M	0.108		0.133		0.121		0.120		0.242*		0.290*		0.647*		0.677*	
	F	0.138		0.221		0.142		0.164		0.267*		0.287		0.840*		0.681*	

* p <0.05, ** p,0.01, Hb = haemoglobin, HCT = haematocrit, MCH = mean cell haemoglobin, ASAT = aspartate aminotransferase, N-dem = N-demethylase, O-dem = O-demethylase, CAT = carnitine acyl transferase

Table A6.5(01) -2. Main toxicological findings observed in male and female rats during the carcinogenicity phase with transfluthrin

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	59	59	60	60	59	60	58	60		
Mortality	2	1	3	8	8	6	4	5	-	-
Clinical signs	-	-	-	-	-	-	-	-		
Body weight	-	-	-	-	↑*	-	↑*	↓*		
Food consumption	-	-	-	-	-	-	-	-		
Overall tumour incidence (%):	44	64	55	72	56	53	62	50		
No. of animals with neoplasms	26/59	38/59	33/60	43/60	33/59	32/60	36/58	30/60	+	-
No. of animals with benign neoplasms	23/59	38/59	22/60	34/60	25/59	33/60	30/58	30/60	+	-
No. of animals with malignant neoplasms	2/59	3/59	4/60	4/60	3/59	3/60	2/58	5/60	-	-
No. of animals with > 1 neoplasm	1/59	3/59	3/60	7/60	0/59	2/60	0/58	2/60	-	-
Liver										
Hepatocellular adenoma	0/59	0/59	3/60	0/60	2/59	0/60	3/58	0/60	-	-
Carcinoma	1/59	0/59	0/60	0/60	0/59	0/60	0/58	0/60	-	-
Non-neoplastic changes										
Swollen/thickened/ enlarged	0/59	0/59	4/60	0/60	0/59	2/59	5/58	3/60	-	-
Nodule	0/59	0/59	3/60	0/60	2/59	0/60	3/58	0/60	-	-
Absolute weight (interim 12 month)	-	-	-	-	-	-	↑**	↑**		
Absolute weight (final 24 month)	-	-	-	-	-	-	↑	↑*		
Kidney										
Tumour (lipomatous)	0/59	0/59	0/60	1/60	0/59	0/60	2/58	0/60		
Carcinoma	0/59	0/59	1/60	0/60	0/59	0/60	0/58	0/60		
Non-neoplastic changes										
Glomerulonephrosis	45/59	11/59	47/60	18/60	53/59	21/60	56/58	13/60		
Pigment deposition	41/59	33/59	41/60	40/60	53/59	54/60	58/58	59/60		
Absolute weight (interim 12 month)	-	-	-	-	↑	↑*	↑*	↑*		
Absolute weight	-	-	-	-	↑**	↑*	↑**	↑		

(final 24 month)										

Continued

Table A6.5(01) -2. continued

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Urinary bladder										
Papilloma	0/58	0/59	0/59	0/60	0/58	0/60	2/57	1/60		
Carcinoma	0/58	0/59	0/59	0/60	0/58	0/60	1/57	2/60		
Non-neoplastic changes										
Hyperplasia	2/59	0/59	1/60	1/60	2/59	2/60	7/58	10/60	+	+
Thyroid										
C-cell adenoma	2/58	3/59	2/60	5/60	1/59	2/60	2/58	2/59	-	-
Follicular adenoma	3/58	1/59	1/60	0/60	1/59	1/60	2/58	1/59	-	-
Follicular adenocarcinoma	0/58	0/59	0/60	1/60	0/59	0/60	1/58	0/59	-	-
Non-neoplastic changes										
Follicular hyperplasia	0/59	0/59	0/60	0/60	3/59	1/60	4/58	2/60	-	-
Increased cuboidal cells	1/10	1/10	2/10	2/10	2/10	1/10	7/10	2/10	-	-

*p <0.05, ** p < 0.01,- Not significantly different than control.

Appendix

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Transfluthrin Tech. – BES's position on the derivation of the Acceptable Exposure Level for systemic chronic exposure

3. Kidney effects observed in the transfluthrin chronic/carcinogenicity study

Transfluthrin long-term effects have been evaluated in a two-year rat study in which animals were treated at 0, 20, 200 and 2000 ppm equivalent to 0, 1.0, 9.9, 100.4 mg/kg bw/day in the males and 0, 1.4, 13.6, 142.1 mg/kg bw/day in the females for 1- (interim sacrifice, 10 animals/sex/group) and 2 years (terminal sacrifice, 60 animals/sex/group; Eiben *et al.*, 1993).

The kidney was reported to be a target organ and findings in this organ are summarised in table 4 below:

Table 4: Histopathology of the kidneys in the rat 2-year chronic/carcinogenicity study [redacted] 1993).

1-year interim sacrifice								
Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**20**	**200**	**2000**	**0**	**20**	**200**	**2000**
Examined kidneys	10	10	10	10	10	10	10	10
Glomerulonephrosis	0	1	2	6	0	0	1	0
Cyst(s)	1	0	0	0	0	0	0	0
Pigment	0	0	2	9	0	0	4	7
Calcification	0	0	0	0	1	2	1	0
2-year terminal sacrifice								
Sex	**Males**				**Females**			
Dose level (ppm)	**0**	**20**	**200**	**2000**	**0**	**20**	**200**	**2000**
Examined kidneys	59	60	59	58	59	60	60	60
Glomerulonephrosis	45	47	53	56	11	18	21	13
Pigment	41	41	53	58	33	40	54	59

Bayer CropScience

Transfluthrin Tech. – BES's position on the derivation of the Acceptable Exposure Level for systemic chronic exposure

After 1-year of treatment, the kidneys showed deposits of yellowish brown pigment in both male and female animals of the 200 and 2000 ppm groups. In addition, males given the high dose exhibited an increased incidence of glomerulonephrotic foci. These findings are considered to demonstrate a degenerative effect of the test substance, resulting in accelerated aging of the kidneys. In the male rats of the high dose group, this was found at an increased occurrence. At terminal sacrifice, an increased incidence of glomerulonephrosis (progressive or senile nephropathy) was observed in the males only from 200 ppm. There was also an increased incidence of pigment (lipofuscin in epithelia of the proximal tubule) from 200 ppm in both sexes.

Therefore, based on these findings, the No Adverse Effect Level (NOAEL) for kidney effects is concluded to be of 20 ppm equivalent to 1.0 and 1.4 mg/kg bw/day in males and females, respectively.

4. Derivation of the AEL for systemic chronic exposure to transfluthrin

The study of choice to derive the AEL for systemic chronic exposure to transfluthrin is the chronic/carcinogenicity study in which long term effects of this active ingredient on target organs including the kidney have been investigated and well characterised. A conventional safety factor of 100 to account for the inter- and intra-species variability (10X and 10X, respectively) is applied since there is no additional concern for tumorigenicity or for pup survival in contrast to Ctgb's proposal, as presented in two other statements provided along with the present one (Lautraite and Wason, 2008a and b).

The AEL value is therefore calculated as follow:

$$\text{AEL}_{\text{systemic chronic exposure}} = \frac{\text{NOAEL from rat 2-year study}}{\text{Safety Factor}} = 0.01 \text{ mg/kg bw/day}$$

Where:
NOAEL from rat 2-year study = 1 mg/kg bw/day
Safety factor = 100

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Official use only

66 REFERENCE

66.1	**Reference**	[redacted] (1993, amended 1999) NAK 4455. Study for oncogenicity in B6C3F1 mice after administration in diet for 2 years. [redacted]. [redacted] Report No. T 9027514 [BES Ref: MO-03-010149] Report date: December 13, 1993 (amended January 7, 1999). Unpublished
66.2	**Data protection**	Yes
66.2.1	Data owner	Bayer CropScience
66.2.2	Companies with letters of access	
66.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

67 GUIDELINES AND QUALITY ASSURANCE

67.1	**Guideline study**	Yes OECD 451 (1981) US EPA FIFRA § 82-2 (1984)
67.2	**GLP**	Yes
67.3	**Deviations**	No

68 MATERIALS AND METHODS

68.1	**Test material**	NAK 4455 (transfluthrin)
68.1.1	Lot/Batch number	Mixed batch no: 250987
68.1.2	Specification	As given in sections 2 and 3

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68.1.2.1	Description	Dark brown
68.1.2.2	Purity	94.5- 95%
68.1.2.3	Stability	Test substance was stored at room temperature in laboratory cabinet and kept stable throughout the study—Stable to May 1990. Test substance was added to the powdered food in accordance with the dose plan for each successive week. Test compound content in the administered formulation was checked at the start of study, and approximately every 3 months thereafter. Stability and homogeneity were tested before beginning of study. Purity of 100% was assumed for the technical test compound, which contained 1% peanut oil to minimize dust generation. The test compound was found to be stable in the diet over 10 days within a tolerance range of 20%, it was found to be homogenous in the diet within a tolerance of 10%. The mean concentration was within 10% of the nominal concentration.
68.2	**Test Animals**	
68.2.1	Species	Mice
68.2.2	Strain	B6C3F1
68.2.3	Source	[redacted]
68.2.4	Sex	Male and female
68.2.5	Age/weight at study initiation	5-6 weeks Weight range at start of study 18-24 g (males) and 15-20 g (females)
68.2.6	Number of animals per group	60 rats/sex/group (+ extra 10 rats/sex/group for 0 and 1000 ppm groups)
68.2.6.1	at interim sacrifice	10 animals/sex/group at 12 months
68.2.6.2	at terminal sacrifice	50 animals/sex/group
68.2.7	Control animals	Yes
68.3	**Administration/ Exposure**	Oral
68.3.1	Duration of treatment	24 months
68.3.2	Interim sacrifice(s)	After 12 months
68.3.3	Final sacrifice	After 24 months
68.3.4	Frequency of exposure	Daily
68.3.5	Postexposure period	None
		Oral
68.3.6	Type	In food
68.3.7	Concentration	Food 0, 10, 100, 1000 ppm, equivalent to:

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		Males: 0, 2.1, 19.7, 199.5 mg/kg bw-day Females: 0, 3.1, 33.3, 279.0 mg/kg bw-day Food consumption per day ad libitum
68.3.8	Vehicle	Moistened with peanut oil/ mixed into food
68.3.9	Concentration in vehicle	Not applicable
68.3.10	Total volume applied	Not applicable
68.3.11	Controls	Diet with peanut oil
68.4	**Examinations**	
68.4.1	Body weight	Yes, before administration of first dose and then weekly.
68.4.2	Food consumption	Yes, measured weekly (based on extra 10 animals).
68.4.3	Water consumption	Yes, measured weekly.
68.4.4	Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
68.4.5	Macroscopic investigations	Palpable masses, skin tumours
68.4.6	Ophthalmoscopic examination	No.
68.4.7	Haematology	Yes, 10 animals/sex/group Time points: after 3 (extra groups only) 12, 18 (only differential blood count), 24 months of treatment Parameters: haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, thrombocyte count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular cell volume (MCV)
68.4.8	Clinical Chemistry	Yes, 10 animals/sex/group Time points: after 3 (extra and main group animals), 12, 24 months of treatment Parameters: glucose, total cholesterol, urea, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate amino-transferase, alkaline phosphatase
68.4.9	Urinalysis	No
68.4.10	Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at 3-month, interim and final sacrifice
68.4.10.1	Organ Weights	Yes, from all animals at 3-month, interim or final sacrifice Organs: liver, kidneys, testes, spleen, brain, heart, ovaries, and lungs
68.4.11	Histopathology	Yes, from all sacrificed animals

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		Organs: fixed in 10% buffered formaldehyde solution (urinary bladder and lungs fixed by instillation): adrenal glands, aorta, bone marrow (femur and sternum), brain (cerebrum, cerebellum, brain stem), cymbal gland, ears (tattooed), epididymides, oesophagus, eyes (including lids and optic nerves), femur with knee joint, gall bladder, Harderian glands, "head" (nasal and oropharyngeal cavity), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum; remaining intestinal tissue), kidneys, lachrymal glands (extraorbital), larynx, liver, lungs, mammary gland, mandibular lymph node, mesenteric lymph node, ovaries (including oviducts), parathyroid glands, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, ureter, urethra, urinary bladder, uterus, vagina and any other tissue showing changes.
68.4.12	Other examinations	Enzyme induction: At 3 months (all extra group animals) and at sacrifice (interim and final) 5 animals/sex/group were examined for enzyme induction in the liver, specifically: N-demethylase and cytochrome P450. Fluoride content: At 3 months (all extra group animals) and at sacrifice (interim and final), the teeth and bones of 5 animals/sex/group were analysed for fluoride levels.
68.5	**Statistics**	Arithmetic group means and standard deviation were calculated for all quantitative results (except fluoride data). Test collective data were compared with control collective data using either Mann and Whitney or Wilcoxon's U-test. Differences were considered significant at the 5% and 1% probability level. Data from the fluoride analysis were evaluated at a confidence level of 0.05. The Box test was used to test for homogeneity of variances between groups. If a difference was seen, a pairwise post-hoc comparison of the groups (one and two-tailed) was made using the Games and Howell modification of the Tukey-Kramer significance test. Comparison of survival curves used Wilcoxon's generalized test (Breslow test), a weighting proportional to respective group sized per event time.
68.6	**Further remarks**	
		69 RESULTS AND DISCUSSION
69.1	**Mortality**	No treatment-related mortality was observed among all groups.
69.2	**Body weight**	No treatment related effects were seen in male animals. Female animals in the high dose group had a slight but significant increase in weight from week 1 to week 83.
69.3	**Food consumption**	No treatment related effects were seen.
69.4	**Water consumption**	No treatment related effects were seen.
69.5	**Clinical signs**	No treatment related effects were seen,
69.6	**Macroscopic investigations**	No treatment related effects were seen.

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69.7	**Ophthalmoscopic examination**	Not applicable
69.8	**Haematology**	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, there is a suggestion of an effect on red cells—erythrocytes were reduced in high dose males, as were haemoglobin levels and haematocrit. Thrombocytes were increased in high dose males and females. There appeared to be no treatment related effect on white cells, with the possible exception of high dose group females at 24 months, which had an increased % of lymphocytes and decreased % of polymorphonuclear neutrophils (PMN).
69.9	**Clinical Chemistry**	A number of miscellaneous statistically significant effects occurred which appear to have no toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, cholesterol levels were significantly higher for high dose group males and females at all time points, for males and females in the 100 ppm dose group at interim sacrifice, and for females in the 100 ppm and 10 ppm dose groups at final sacrifice without clear dose-relationship. Additionally, protein and albumin levels were significantly increased for females in all treatment groups at final sacrifice. Alkaline phosphatase was significantly increased in high dose groups at all time points.
69.10	**Urinalysis**	Not applicable.
69.11	**Pathology**	Moribund, 3-month and Interim autopsy: No treatment related effects were found. Final autopsy: Incidence of liver nodules was increased in females in the high dose group; no other treatment related effects were seen.
69.12	**Organ Weights**	Absolute and relative liver weights were increased in males and females in the high dose groups. While other statistically significant changes were seen, they appear to have no toxicological significance as there is no apparent dose- or time-response.
69.13	**Histopathology**	Interim autopsy: Hypertrophy of periacinal hepatocytes was seen in all males and more than half of the females in the high dose group. No other treatment related effects were seen. Final autopsy: Hypertrophy of periacinal hepatocytes was seen in more than half of the males and females in the high dose group. No other treatment related effects were seen.
69.14	**Other examinations**	Enzyme induction: Significantly high CYP 450 content and N-demethylase activity were seen in high dose females at week 14. Fluoride incorporation: Fluoride levels in bones and teeth of male animals were statistically significantly increased in the 100 and 1000 ppm groups at both 12 and 24 months and in females animals in the 1000 ppm group.

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69.15	**Time to tumours**	Not applicable
69.16	**Other**	Neoplastic lesions: No treatment related neoplastic lesions were seen at the interim autopsy. At the 24-month final autopsy a miscellany of benign and malignant tumours were seen an all groups (including controls) and were clearly not treatment related (lack of dose response, increased incidence in controls, single instance in middle dose group, etc). Female animals in the high dose group had a statistically significantly increased number of hepatocellular adenomas. Because of this, females in the high dose group also had a higher number of total and benign tumours.

70 APPLICANT'S SUMMARY AND CONCLUSION

70.1	**Materials and methods**	Groups of 60 male and female B6C3F1 mice were given NAK 4455 in the diet at concentrations of 0, 10, 100 and 1000 ppm for 12 months at which point 10 rats/sex/group were sacrificed (interim autopsy). The remaining 50 rats/sex/group were given NAK 4455 in the diet for an additional 12 months before sacrifice. Additionally, a further 10/animals/sex were treated for 13 weeks with either 0 or 1000 ppm NAK4455. Haematology, clinical chemistry, liver enzyme induction, measurement of fluoride levels, and gross and histopathology were performed on all animals at or just before sacrifice. Additionally, haematology and clinical chemistry and urinalysis were performed at 12, 18 (differential blood count only) and 24 months. This study fulfils the requirements of OECD 451 (1981) and US EPA FIFRA § 82-2 (1984), with the exception that an ophthalmoscopic examination was not performed.
70.2	**Results and discussion**	Mortality was unaffected by treatment. No treatment induced changes in behaviour, or appearance were observed. No treatment related effects were seen on food or water consumption. Body weights of females in the high dose group were statistically significantly increased (≤ 10%) over controls except during the last part of the study. The results from the haematological and clinical chemistry studies combined with histopathology suggest that liver damage occur in both sexes exposed to 1000 ppm and may begin at 100 ppm in females. In the high dose group, liver weights were increased and increased cholesterol levels were seen. In female animals increased incidence of liver nodes was seen. In week 14, females in the high dose group had significantly increased levels of N-demethylase activity and CYP 450 content, suggesting liver effects. In male and female animals, increased hypertrophy of periacinal hepatocytes was seen at interim and final autopsy. High dose group females had increased levels of polymorphonuclear neutrophils, suggesting organ inflammation. High dose group females had increased levels of hepatocellular adenomas. This is not surprising given the liver damage that is apparently occurring at 1000 ppm and likely represent an epigenetic mechanism.

		Also in the high dose group, there was an apparent decrease in haemoglobin and erythrocytes, particularly in male animals at interim sacrifice. Both males and females had increased thrombocytes in the high dose group. A dose dependent increase in fluoride content in teeth and bones was seen starting at 100 ppm. The lowest adverse effect level in this study is 100 ppm (equivalent to approximately 19.7 and 33.3 mg/kg bw-day for males and females respectively) based on liver damage in both sexes. The no observable adverse effect level is 10 ppm (equivalent to approximately 2.1 and 3.1 mg/kg bw-day for males and females respectively). This compound appears to cause benign liver adenomas in female animals at the 1000 ppm dose level (equivalent to 279 mg/kg-day). Based on the clear lack of a genotoxic mechanism, the propensity of mice to develop hepatoadenomas, and the lack of this response in another species, this compound does not present a carcinogenic risk to humans.
70.3	**Conclusion**	The lowest adverse effect level in this study is 100 ppm (equivalent to approximately 19.7 and 33.3 mg/kg bw-day for males and females respectively) based on liver damage in both sexes. The no observable adverse effect level is 10 ppm (equivalent to approximately 2.1 and 3.1 mg/kg bw-day for males and females respectively). There is no indication of a carcinogenic risk to humans.
70.3.1	Reliability	1
70.3.2	Deficiencies	None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24 january 2007
Materials and Methods	The version of the applicant is acceptable. Note: Purity of the compound is 94.4-95%. For the 3-month extra group histopathology was only performed on liver. For the 12-month interim kill histopathology was only performed on kidneys, liver, thyroid and parathyroid , and altered organs and tissues.
Results and discussion	It is noted that in females of the high dose the incidences of haemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50) and sarcomas of the subcutis (2/50) were increased. The incidences are above the historical control range. Otherwise the version of the applicant is adopted. 22-3-2011; Data in DOCIIIA 6.5/02 deviate from the original report, they have been mixed up. The right data are presented under table A6.5(02) -2 .
Conclusion	Based on the observed changes in haematology and clinical chemistry the LOAEL was 100 ppm, equal to 19.7mg/kg bw/day. The NOAEL is 10 ppm, equal to 2.1 mg/kg bw/day. In the females at 1000 ppm there may be a treatment-related increase in haemangiosarcomas in the spleen, adenomas in the Harderian gland and sarcomas of the subcutis. The incidences of these neoplastic lesions are above the historical control range and are considered possibly related to treatment. 22-3-2011: Based on new data the conclusion is adjusted, see discussion in Doc IIA.
Reliability	1
Acceptability	acceptable
Remarks	Although the study is described as a carcinogenicity study (OECD 451) a number of additional parameters have been studied.

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.5(02) -1. Main haematological and clinical chemistry findings observed in male and female mice during long term exposure to transfluthrin

Affected		0 ppm				10 ppm				100 ppm				1000 ppm			
Parameter, unit, sex		Months after start of treatment															
Haematology		3	12	18	24	3	12	18	24	3	12	18	24	3	12	18	24
Ery, 10^{12}/L	M	9.32	9.77		9.79		9.76		9.73		9.76		10.13	9.10**	9.37**		9.58
	F	8.67	9.42		8.46		9.69*		9.46		9.59		9.12	8.93	9.47		9.22
Hb, g/L	M	165	151		148		151		151		153		152	157**	149		146
	F	161	148		133		150		145		149		138	157	146		141
HCT, L/L	M	0.475	0.465		0.455		0.463		0.457		0.455		0.466	0.457**	0.438**		0.446
	F	0.472	0.422		0.406		0.457*		0.450		0.454		0.423	0.459*	0.446		0.435
MCH, pg	M	17.7	15.5		15.1		15.5		15.5		15.7		15.1	17.3*	15.9**		15.3
	F	18.6	15.7		15.9		15.5		15.4		15.6		15.2	17.6	15.5		15.3*
MCHC, g/L ery	M	348	325		325		327		330*		337**		326	344**	340**		329*
	F	341	335		327		329*		325		329**		327	341	328**		325
Thromboc ytes, 10^9/L	M	911	891		1212		910		1271		907		1382**	980*	947		1425**
	F	763	779		658		852*		846**		823		741	880*	899**		871**
Lymphocyt es, %	M	79.1	75.3	70.9	69.4		76.1	71.2	66.0		79.4	68.9	63.6	82.3	77.1	69.2	66.4
	F	83.1	76.6	69.2	69.5		82.1	71.1	72.2		83.0*	70.7	71.6	87.9*	81.8	74.5	86.8**
PMN, %	M	18.9	17.5	26.5	27.8		17.3	24.7	29.9		16.5	27.7	31.4	17.0	16.0	28.1	31.7
	F	15.5	17.0	26.3	27.3		12.3	24.2	22.5		12.8*	21.6	23.6	11.1	12.5*	20.6	11.1**
Clinical Chemistry																	
Months		3	12	24		3	12	24		3	12	24		3	12	24	
APh, U/L	M	128	87	105		130	86	103		126	92	119		137*	104**	139**	
	F	210	171	340		199	155	367		190	166	389		219	205**	741**	
Cholesterol, mM	M	2.87	3.22	3.08		2.85	2.98	3.02		2.97	3.58*	3.56		3.37**	3.69*	3.71*	
	F	2.36	2.16	2.17		2.52	2.38	3.41**		2.51	2.63**	3.72*		2.89**	2.85**	3.35**	
Protein, g/L	M	51.3	54.3	55.8		49.7	54.9	55.5		50.5	55.6	59.5		51.7	54.7	57.9	
	F	50.5	54.0	52.5		50.1	54.5	58.6		50.3	55.4	58.1*		51.0	55.2	58.3**	
Albumin, g/L	M	24.3	25.2	26.2		22.9	25.7	26.4		24.1	25.6	26.8		25.2	26.0	27.2	
	F	26.4	27.5	25.6		26.3	27.7	29.3**		26.6	28.4	28.8**		27.1	27.3	29.7**	
Fluoride, teeth, mg/g	M	0.259	0.494	0.584			0.538	0.651			0.798*	1.112**		1.301[a]	2.352**	2.349**	
	F	0.288	0.480	0.578			0.379	0.606			0.613	0.875		1.192[a]	2.032**	2.269**	
Fluoride, bone, mg/g	M	0.567	0.863	1.014			0.866	1.061			1.476**	1.824**		2.899[a]	4.428**	5.305**	
	F	0.506	0.790	0.962			0.783	1.098			1.338**	1.697		2.194[a]	3.843**	4.292**	

* p <0.05, ** p<0.01, [a] No statistical analyses performed for "extra groups" fluoride content, Ery = erythrocytes, Hb = haemoglobin, HCT = haematocrit, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, PMN = polymorphonuclear neutrophils, APh = alkaline phosphatase

Table A6.5(02) -2. Main toxicological findings observed in male and female mice during the carcinogenicity phase with transfluthrin

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	50	50	50	50	50	50	50	50		
Mortality	9	5	2	2	8	11	8	6	-	-
Clinical signs	-	-	-	-	-	-	-	-	-	-
Body weight	-	-	-	-	-	-	-	↑**	-	-
Food consumption	-	-	-	-	-	-	-	-	-	-
Overall tumour incidence (%):	50	58	42	54	50	56	40	74		
No. of animals with neoplasms	25/50	29/50	21/50	27/50	25/50	28/50	20/50	37/50	-	-
No. of animals with benign neoplasms	14/50	13/50	10/50	14/50	11/50	9/50	9/50	19/50	-	-
No. of animals with malignant neoplasms	10/50	10/50	9/50	11/50	11/50	16/50	7/50	10/50	-	-
No. of animals with > 1 neoplasm	3/50	9/50	3/50	5/50	6/50	6/50	7/50	17/50	-	-
Liver										
Hepatocellular adenoma	5/49	4/50	5/50	5/50	4/50	2/48	2/50	13/50*	-	-
Carcinoma	5/49	8/50	7/50	7/50	2/50	2/48	4/50	4/50	-	-
Non-neoplastic changes										
Nodule	10/50	7/50	13/50	4/50	13/50	5/50	12/50	15/50	-	-
Hypertrophy of periacinal hepatocytes (interim)	0/10	0/10	0/10	0/10	0/10	0/10	10/10	6/10	-	-
Hypertrophy of periacinal hepatocytes (final)	0/50	0/50	0/50	0/50	0/50	0/50	38/50	26/50	-	-
Absolute weight (interim 12 month)	-	-	-	-	-	-	↑**	↑**	-	-
Absolute weight (final 24 month)	-	-	-	-	↑*	↑	↑**	↑**	+	+

*p <0.05, ** p<0.01,- Not significantly different than control.

Right data for hepatocellular adenoma and liver carcinoma

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Liver										
Hepatocellular adenoma	5/49	4/50	4/50	2/48	5/50	2/50	5/50	13/50*	-	-
Carcinoma	5/49	2/50	8/50	2/48	7/50	4/50	7/50	4/50	-	-

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71 REFERENCE

71.1	**Reference**	[redacted] (1993); NAK 4455 Chronic toxicity study in dogs (52-week feeding study), [redacted], unpublished report no. 22638, 28 October 1993. [redacted] Study number: T 6030481. Histopathological Examination, [redacted] Dates of work: December 1988 to December 1989. [BES Ref No: MO-03-010104]
71.2	**Data protection**	Yes
71.2.1	Data owner	BAYER AG
71.2.2		
71.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

72 GUIDELINES AND QUALITY ASSURANCE

72.1	**Guideline study**	Yes OECD Guidelines 452 (1981)
72.2	**GLP**	Yes
72.3	**Deviations**	None which affected the integrity of the study.

73 MATERIALS AND METHODS

73.1	**Test material**	NAK 4455 (transfluthrin)
73.1.1	Lot/Batch number	250987
73.1.2	Specification	As given in sections 2 and 3
73.1.2.1	Description	Solid
73.1.2.2	Purity	95.1%
73.1.2.3	Stability	Stability and homogeneity and content of test article in feed were verified by analysis before beginning of study. Content was verified regularly during study.
73.2	**Test Animals**	
73.2.1	Species	Dog
73.2.2	Strain	Beagle
73.2.3	Source	[redacted]
73.2.4	Sex	Male and female

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73.2.5	Age/weight at study initiation	21-25 weeks old 5.9-9.7 kg
73.2.6	Number of animals per group	4 dogs/sex/group
73.2.7	Control animals	Yes
73.3	**Administration/ Exposure**	Oral (dietary)
73.3.1	Duration of treatment	1 year (364 or 365 days)
73.3.2	Frequency of exposure	Daily
73.3.3	Postexposure period	None
73.3.4	**Oral**	
73.3.4.1	Type	In food
73.3.4.2	Concentration	In food: 0, 30, 300, and 3000 ppm equivalent to 0, 1, 10, 100 mg/kg bw/day Food consumption per day (offered): 300 g (week 1-9), 350 g (week 10-24) 380 g (week 25-52)
73.3.4.3	Vehicle	Test compound mixed with pulverised dry feed and moistened with water
73.3.4.4	Concentration in vehicle	Not applicable
73.3.4.5	Total volume applied	Not applicable
73.3.4.6	Controls	Pulverised dry feed moistened with water
73.4	**Examinations**	
73.4.1	Observations	Animals were inspected several times daily.
73.4.1.1	Clinical signs	Yes, in addition to general health which was observed daily, reflexes, body temperature and pulse rate were measured in weeks -2, 3, 7, 13, 20, 26, 39, 52
73.4.1.2	Mortality	Yes, observed daily
73.4.2	Body weight	Yes, weekly.
73.4.3	Food consumption	Yes, recorded daily.
73.4.4	Water consumption	Not recorded
73.4.5	Ophthalmoscopic examination	All animals' eyes were indirectly examined with an ophthalmoscope in weeks -1, 7, 13, 26, 39 and 52. The fundus was photographed in weeks -1 and 52.
73.4.6	Haematology	Yes, in weeks -2, 3, 7, 13, 20, 26, 39 and 52. Blood samples were

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		drawn from the jugular vein after light compression into evacuated blood collection tubes coated with EDTA. Parameters: Haematocrit, haemoglobin, mean cell haemoglobin concentration, mean haemoglobin content of erythrocytes, erythrocyte count, total and differential leukocyte count, mean corpuscular volume of erythrocytes, thrombocyte count, reticulocyte count, methaemoglobin, blood corpuscle sedimentation rate and thromboplastin time and partial thromboplastin time.
73.4.7	Clinical Chemistry	Yes, in weeks -2, 3, 7, 13, 20, 26, 39 and 52. Additionally ASAT, ALAT, APh, and GLDH were measured in weeks 2 and 5. Blood samples were drawn from the jugular vein after light compression. Determinations were carried out with blood plasma—except for electrolytes which were measured in serum, and glucose (whole blood). For this purpose, blood was placed in heparin coated tubes. Parameters: glucose, cholesterol, urea, bilirubin, creatinine, total protein, alanine aminotransferase (ALAT/GPT), aspartate aminotransferase (ASAT/GOT), alkaline phosphatase (APh), glutamate dehydrogenase (GLDH), sodium, potassium, chloride, calcium, inorganic phosphate, magnesium, triglycerides (in plasma and in liver tissue), lactate dehydrogenase, creatine kinase, triiodothyronine (T3), thyroxine (T4), thyroxine binding capacity, serum protein electrophoresis and in liver tissue (sampling methodology not stated): Cytochrome P450, N-demethylase, O-demethylase.
73.4.8	Urinalysis	Yes, in weeks -2, 3, 7, 13, 20, 26, 39 and 52. Animals were individually places in metabolism cages from approximately 8.00-14.00 hours without access to food or water. Before being placed in urine collection cage, animals were administered approx. 250 mL tap water by stomach tube. Parameters: volume, specific gravity, protein, glucose, blood, ketone bodies, bilirubin, urobilinogen, and after sedimentation: epithelia, erythrocytes, leukocytes, bacteria and crystals
73.5	**Sacrifice and pathology**	All animals were sacrificed by exsanguination at the end of the study under Evipan anaesthesia, dissected and grossly examined.
73.5.1	Organ Weights	The following organs were weighed: liver, kidneys, adrenals, testes, spleen, brain, heart, ovaries, pancreas, prostate gland, thyroid gland, pituitary and lung.
73.5.2	Gross and histopathology	Samples (fixitive not stated) of the following organs and tissues were examined histopathologically: Abnormalities, adrenals, aorta, bone marrow, brain, cecum, colon, duodenum, epididymides, eyes/optic nerves, femur with marrow, gall bladder, heart (ventricle to include papillary muscle), ileum, jejunum, kidneys, liver, lungs, lymph node (mesenteric), oesophagus, ovaries, pancreas, pituitary, prostate, rectum, salivary gland, sciatic nerve, skeletal muscle, skin with mammary region, spinal cord, spleen, sternum, stomach, testes, thymus, thyroids with parathyroids, tonsil, tongue, trachea, urinary bladder, uterus. Sections from fundic and pyloric regions of the stomach and two pieces

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		of ventricle, including papillary muscle, from the heart were prepared. Two sections of the liver and one of the lung were similarly processed. Sections from the cervical, thoracic and lumbar regions of the spinal cord and three sections of the brain were also taken. All abnormalities were sectioned. After dehydration in alcohol and embedding in paraffin wax, sections were cut at approximately 5 micron thickness and stained with haematoxylin and eosin. An additional piece of liver was frozen, sectioned and stained with Oil Red '0'. An additional piece of kidney was stained using the PAS technique. The tissue sections were each examined by light microscopy and salient features entered onto the Xybion Path/Tox System.
73.5.3	Other examinations	None
73.5.4	Statistics	Descriptive statistics, including mean and standard deviation, maximum and minimum, were produced. However, measures of statistical significance were not produced for any examination except histopathology. Fisher's Exact Probability Test was applied as a two-tailed test, to the distribution of macroscopic or microscopic (non-neoplastic) pathological entities.
73.6	**Further remarks**	
		74 RESULTS AND DISCUSSION
74.1	**Observations**	
74.1.1	Clinical signs	No treatment related effects on general health, reflexes, temperature or pulse were observed.
74.1.2	Mortality	No mortalities were seen.
74.2	**Body weight**	There was no treatment related effect on body weight.
74.3	**Food consumption and compound intake**	No treatment related effects were seen on food consumption or compound intake.
74.4	**Ophthalmoscopic examination**	No treatment related effects.
74.5	**Blood analysis**	
74.5.1	Haematology	There were no treatment related effects.
74.5.2	Clinical chemistry	ALAT was occasionally increased in all treated animals as was alkaline phosphatase, GLDH, and cholesterol. Plasma triglycerides were elevated in the top dose group. Bilirubin was decreased in the top dose group. However clinical chemistry was performed multiple times over the 52 week experimental period and results for these endpoints varied greatly from one timepoint to the next. These changes do not appear to be treatment related. Hepatic N-demethylase was elevated in top-dose animals and liver triglyceride levels were decreased in all treated animals although this does not appear to be of toxicological significance. Graphical representations of these endpoints are shown in

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		figures A6_5.3-1 through A6_5.3-8.
		At the low dose level, one animal (468) showed a gradual and progressive increase in ALAT, Alkaline Phosphatase and GLDH from approximately week 7 of treatment. Given a lack of temporal similarity with treatment-related effects at higher doses, and an absence of hepatic pathology (even though high enzyme levels persisted to necropsy) the significance of this observation is unclear.
74.5.3	Urinalysis	No treatment related effects were seen.
74.6	**Sacrifice and pathology**	
74.6.1	Organ weights	Absolute and relative liver weights were elevated in all treated males and in females in the 300 and 3000 ppm dose groups. Additionally, relative organ weights of kidney, spleen, thyroid, and adrenals were elevated over controls, but noted by study director to be within range of historical controls.
74.6.2	Gross and histopathology	No treatment related findings were noted.
74.7	**Other**	None

75 APPLICANT'S SUMMARY AND CONCLUSION

75.1	**Materials and methods**	Groups of 4 male and female beagle dogs were given NAK 4455 for one year in diet at doses of 0, 30, 300 and 3000 ppm. All dogs were sacrificed at the end of treatment. Haematology, clinical chemistry, urinalysis, liver enzyme induction, and gross and histopathology were performed on all animals. This study fulfils requirements OECD 452.

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75.2	**Results and discussion**	No treatment induced changes in behaviour, appearance, mortality, body weight, food or compound intake was observed. No treatment related damage to the eye was observed. No treatment related results were seen in haematology or urinalysis studies. No histopathological change was was seen in any treated animal. The results from the clinical chemistry studies suggest that NAK 4455 may have effects on the liver at the top dose. The study director notes that in all treated animals ALAT, a non-specific marker of liver damage was increased, as was AP and GLDH and cholesterol. However, as can be seen in figures A6_5.3-1 through A6.5.3_6, these changes do not appear to be dose or time responsive and do not appear to reflect a toxicologically adverse effect except at the top dose—3000 ppm. Additionally, N-demethylase levels were elevated in top dose animals and bilirubln levels were decreased supporting the indication of liver effects. Figure A6_5.3-8 shows liver triglyceride levels at time of sacrifice. All treated animals have reduced liver triglyceride levels, there is no indication of dose-response, and given the normal variability in liver triglycerides and the absence of gross or histopathological changes in the liver, this effect does not have toxicological significance. In the higher dose groups, relative and absolute liver weights were increased; no histopathological change was in seen in the liver or any other organ in treated animals. The lowest adverse effect level in this study is 3000 ppm (equivalent to approximately 100 mg/kg bw-day) based on changes in clinical chemistry and changes in liver weight. The no observable adverse effect level is 300 ppm (equivalent to approximately 10 mg/kg bw-day). Based on the results of this study, the General Classification and Labelling Requirements for Dangerous Substances and Preparations, as stated in Annex IV to Commission Directive 93/21/EC, indicate that no classification is necessary.
75.3	**Conclusion**	
75.3.1	LO(A)EL	The lowest adverse effect level in this study is 3000 ppm (100 mg/kg bw/day) based on clinical chemistry indicating liver effects in both sexes and increases in liver weight.
75.3.2	NO(A)EL	The no observable adverse effect level in this study is 300 ppm (10 mg/kg bw/day).
75.3.3	Other	
75.3.4	Reliability	1
75.3.5	Deficiencies	None

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Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25 January 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted. The conclusion of the applicant that the (inconsistent) effects on liver enzymes at the low and mid dose do not appear to reflect a toxicologically adverse effect is supported by the 3-month study in the dog in which no effects on liver enzymes was observed at doses up to and including 2500 ppm.
Conclusion	LOAEL: 3000 ppm, equivalent to 100 mg/kg bw/day on the basis of liver effects NOAEL:300 ppm, equivalent to 10 mg/kg bw/day
Reliability	2
Acceptability	acceptable
Remarks	Although the study author concludes that there none of the treatment groups was without substance-induced effects, the RMS considered that the effects observed at 30 and 300 ppm were not adverse.
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6_5.3-1. Main findings observed in male and female dogs after a chronic exposure to transfluthrin

Parameter	0 ppm		30 ppm		300 ppm		3000 ppm	
	m	f	m	f	m	f	m	f
Number of animals examined	4	4	4	4	4	4	4	4
Mortality	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Clinical signs	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
Liver								
Absolute weight, g	387.8	390.3	440.0	394.5	451.5	408.8	569.0	545.5
Relative weight, g/kg	34.45	36.72	40.05	34.10	39.17	41.55	51.52	52.12

All figures below are means for 4 dogs/sex/group

Figure A6_5.3-1 ALAT levels in dogs after chronic NAK 4455 administration

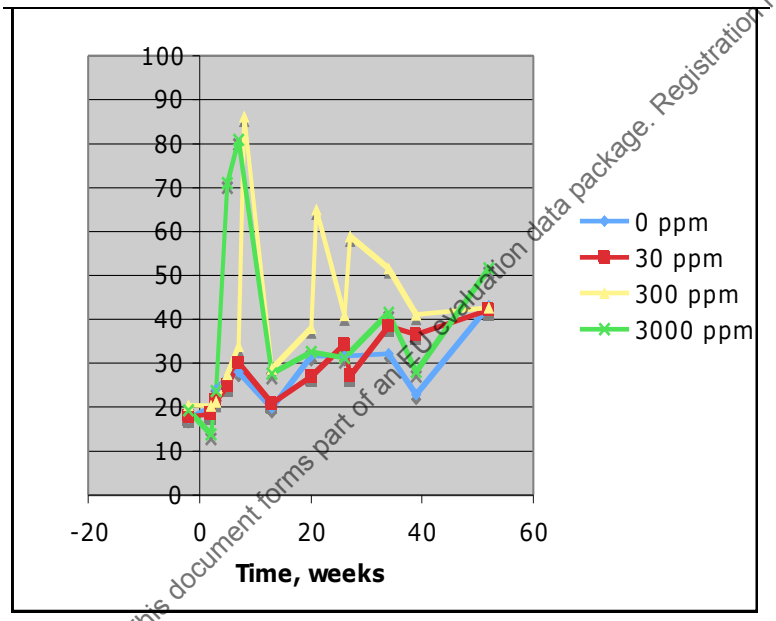


Figure A6_5.3-2 APh levels in dogs after chronic NAK 4455 administration

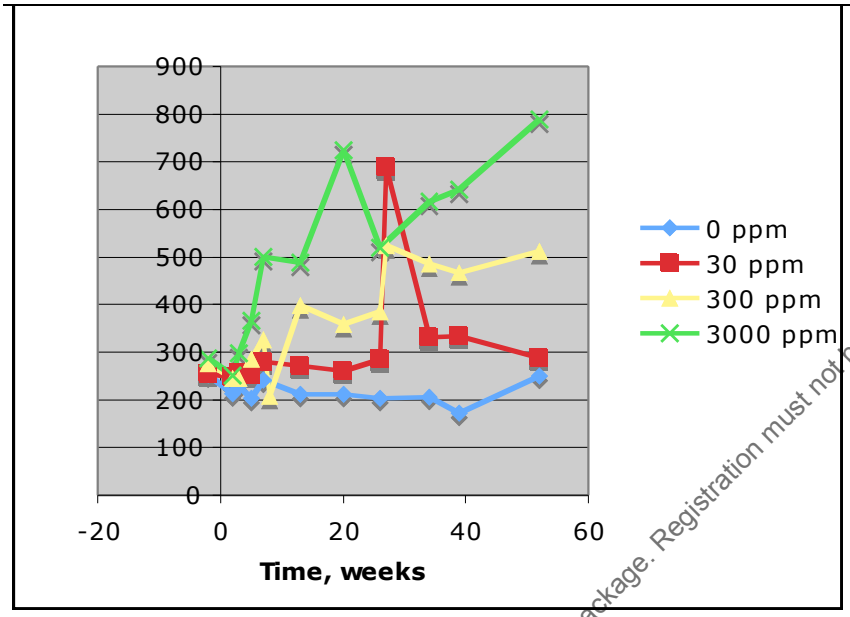


Figure A6_5.3-3 GLDH levels in dogs after chronic NAK 4455 administration

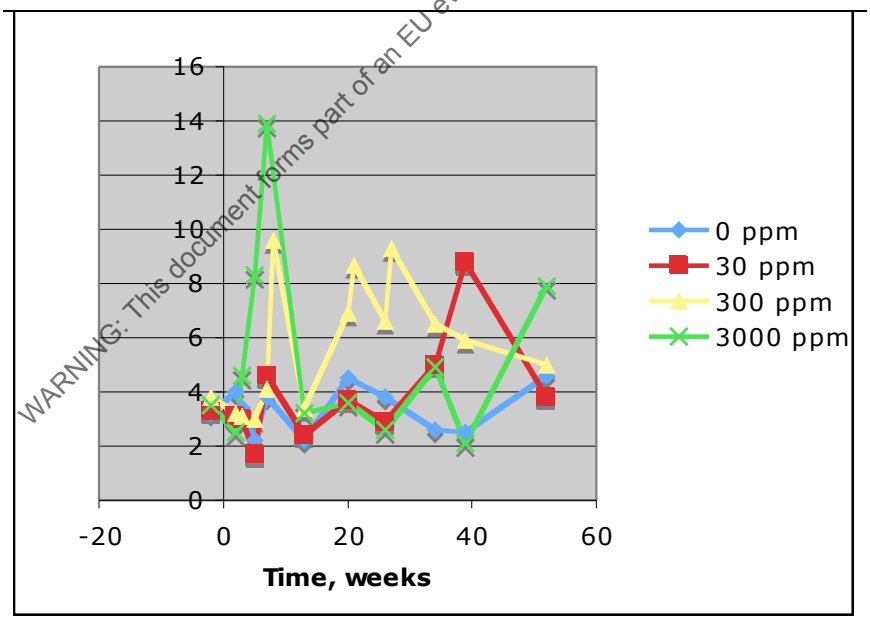


Figure A6_5.3-4 Cholesterol levels in dogs after chronic NAK 4455 administration

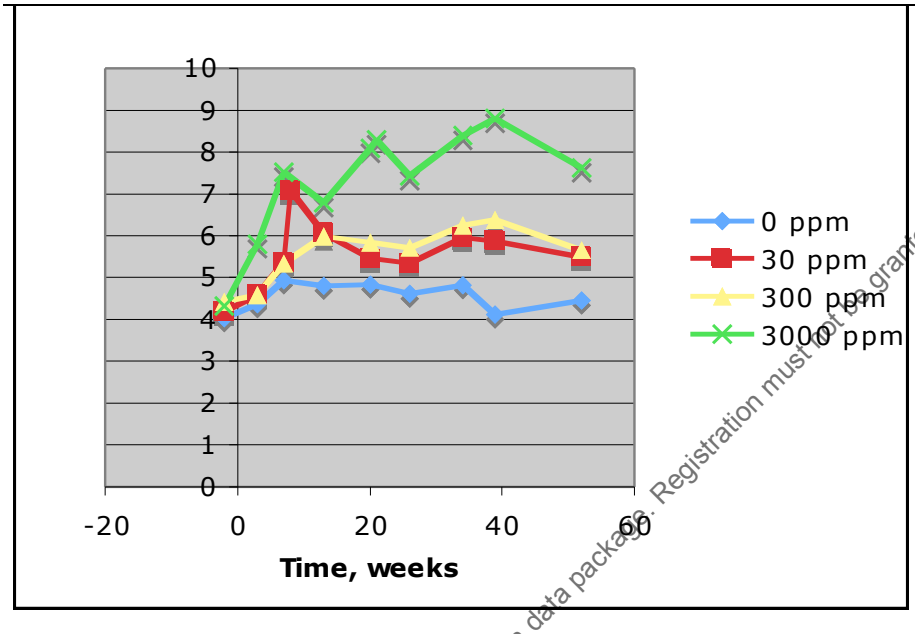


Figure A6_5.3-5 Serum triglyceride levels in dogs after chronic NAK 4455 administration

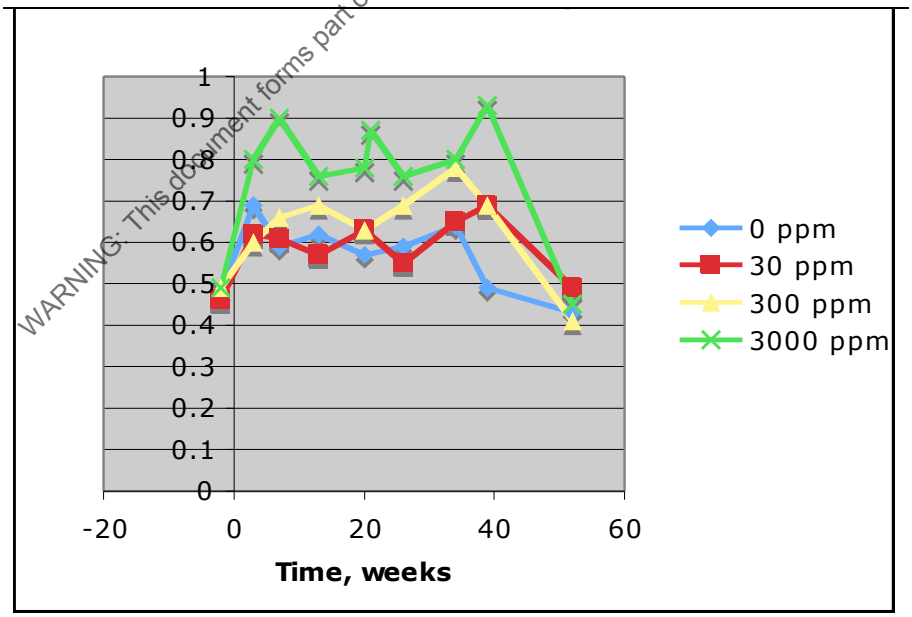


Figure A6_5.3-6 Bilirubin levels in dogs after chronic NAK 4455 administration

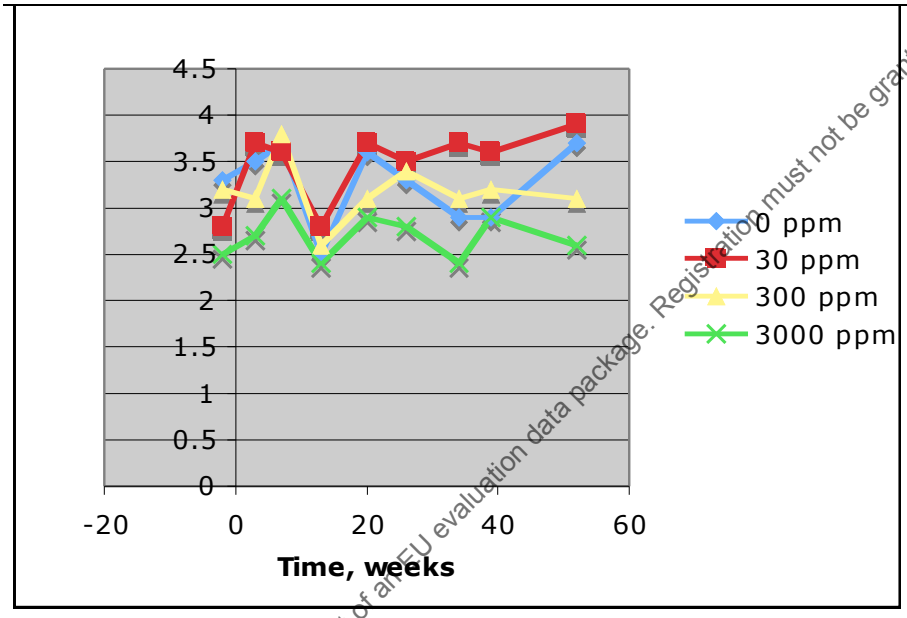


Figure A6_5.3-7 Hepatic N-demethylase levels in dogs after chronic NAK 4455 administration for 52 weeks

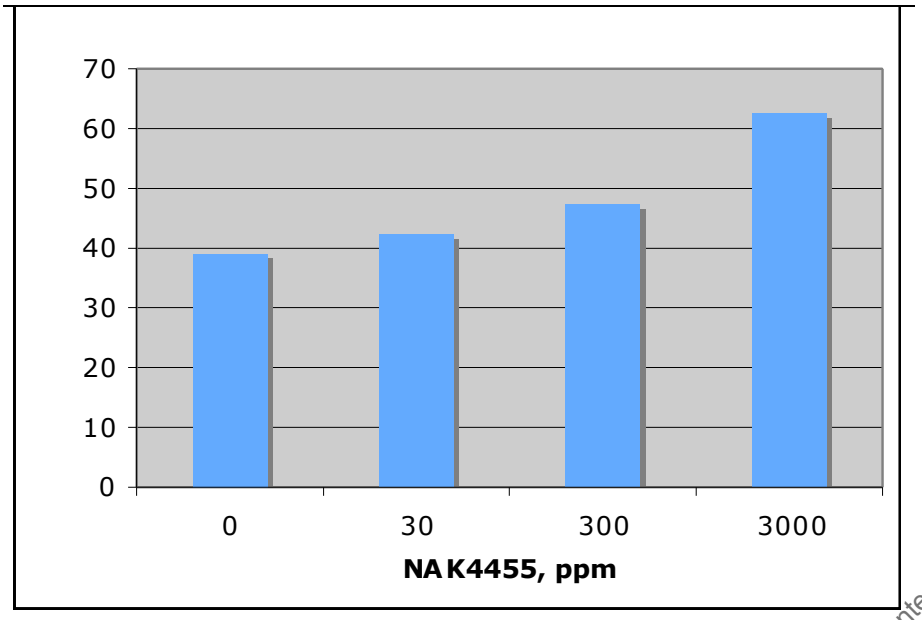
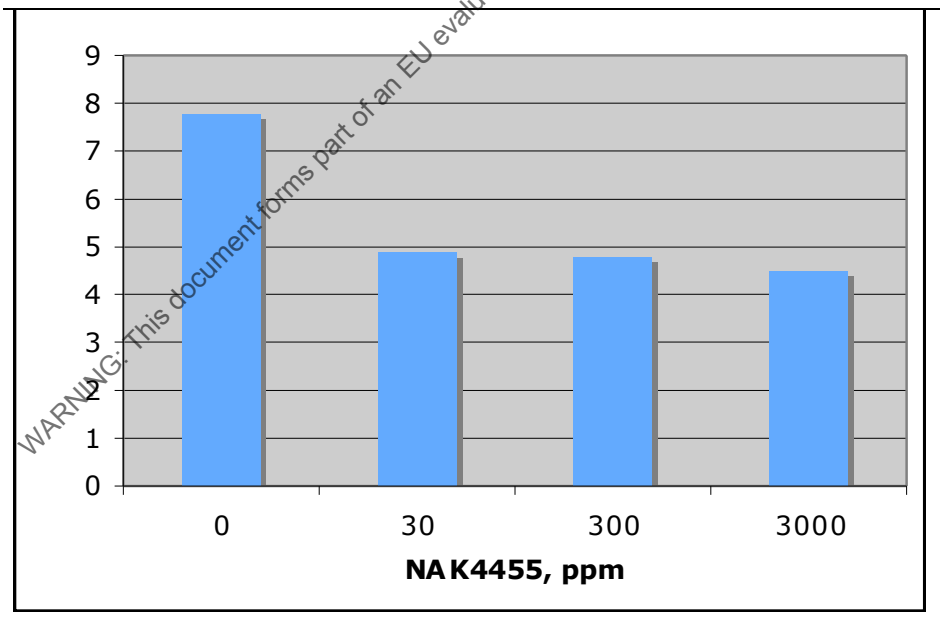


Figure A6_5.3-8 Liver triglyceride levels in dog after chronic NAK 4455 administration for 52 weeks



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Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* and *E.coli* reverse mutation assay)

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		76 REFERENCE
76.1	**Reference**	[redacted] (1987); Salmonella/microsome test to evaluate for point-mutagenic effect, [redacted] [redacted] Report No. T 1024924 [BES Ref: MO-03-009702] Report date: March 19, 1987 Unpublished
76.2	**Data protection**	Yes
76.2.1	Data owner	Bayer CropScience
76.2.2	Companies with letters of access	
76.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I
		2 GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	Yes. Methods used are comparable to OECD 471 "Bacterial Reverse Mutation Test" (1997)
76.3	**GLP**	Yes
76.4	**Deviations**	This study lacks the fifth strain of bacteria recommended to detect AT reversion, such as E. coli WP2 uvrA or S. Typhimurium TA102.
		3 MATERIALS AND METHODS
3.1	**Test material**	NAK 4455, common name transfluthrin
3.1.1	Lot/Batch number	Batch No. 130187
3.1.2	Specification	As given in Section 2 of Doc IIIA
3.1.2.1	Description	Dark brown liquid stored at room temperature
3.1.2.2	Purity	94.5%
3.1.2.3	Stability	The sample used was analysed and approved for a minimum of the test period. A stability test in the solvent did not detect any relevant indication of a change in the active substance (a.s.).
3.2	**Study Type**	Bacterial reverse mutation test
3.2.1	Organism/cell type	<u>*S. typhimurium*</u> *:* TA 1535, TA 1537, TA 98, TA 100. Four plates were used per strain, substance (with or without S9 mix) and dose. All bacterial strains were tested for histidine dependence, ampicillin resistance, crystal violet sensitivity, and UV sensitivity to confirm suitability of the stocks.

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Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* and *E.coli* reverse mutation assay)

3.2.2	Deficiencies / Proficiencies	No E. coli or S. Typhimurium TA102 strains were used to detect cross-linking mutagens.
3.2.3	Metabolic activation system	S9-mix used: Animal species: Male Sprague-Dawley rats, body weight 200-300g Organ: Liver Induction: Yes, single i.p. injection of Aroclor 1254 in corn oil (500 mg/kg)
3.2.4	Positive control	The following substances were used: sodium azide (Na-Az), positive control for TA 1535 strain during non-activation conditions nitrofurantoin (NF), positive control for TA 100 strain during non-activation conditions 4-nitro-1,2-phenylene diamine (4-NPDA), positive control for TA 1537 and TA 98 strains during non-activation conditions 2-amino-anthracene (2-AA) positive control for all strains during activation from the S9 mix
3.3	**Administration / Exposure; Application of test substance**	
3.3.1	Concentrations	An initial test was run with transfluthrin ranging from 20 – 12500 µg/plate. Based on the detected cytotoxicity in the initial tests, doses chosen for the second set of mutagenicity tests fell between 775 and 12400 µg/plate. Positive controls: Na-Az - 10 µg/plate, NF - 0.2 µg/plate, 4-NPDA – 0.5 or 10 µg/plate, and 2-AA – 3 µg/plate.
3.3.2	Way of application	Solutions of all substances were prepared in dimethylsulfoxide (DMSO). All components of each plate were mixed and plated at the start of the incubation period.
3.3.3	Pre-incubation time	None.
3.3.4	Number of replicates	Each assay condition was replicated 4 times simultaneously and the results averaged for reporting in the study. The averaged numbers are given in Table 6.6.1-01.
3.3.5	Confirmatory assay	Table 6.6.1-01 reports data for each dose each time that dose was used in the assay. First test: 0, 10, 20, 100, 500, 2500 12,500 µg/plate. Due to a.s. toxicity, doses chosen for a repeat test ranged from 775 to 12,400 µg/plate. Second test: 0, 775, 1550, 3100, 6200, 12,400 µg/plate. Confirmatory third test: 0, 775, 1550, 3100, 6200, 12,400 µg/plate.

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SECTION A6.6.1

BPD Data set IIA/ Annex Point VI.6.1

Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* and *E.coli* reverse mutation assay)

3.3.6	Other modifications	Initial results were confirmed by a second, and at some concentrations a third, assay.
3.4	**Examinations**	
3.4.1	Number of cells evaluated	Substance toxicity was assessed in three ways. First, background growth on the plates was assessed and indicated if it was reduced. Second, the substance was considered toxic if the count of mutants per plate was clearly lower than the control in a dose-responsive manner. Third, a titre of total bacterial count was determined. A test result was evaluable if the positive and negative control values fell within the expected range according to the literature and historical experience of the lab. A result for transfluthrin was considered evaluable if it provided indications of mutagenesis even while not satisfying standard criteria, and the test was repeated by at least two further tests. A reproducible dose-related increase in mutant counts for at least one strain is considered positive, and about double the negative control count should be reached. If there was no reproducible dose-related increase for at least one strain, the test was considered negative.
		RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
4.2	**Cytotoxicity**	Yes; doses up to 500 µg/plate did not lead to bacteriotoxic effects. Doses above this had bacteriotoxic effects specific to each strain.

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SECTION A6.6.1

BPD Data set IIA/ Annex Point VI.6.1

Genotoxicity in vitro

In-vitro gene mutation study in bacteria (*Salmonella* and *E.coli* reverse mutation assay)

APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

Transfluthrin, batch 130187 at 94.5% purity, was dissolved in DMSO vehicle and added to test strains of bacteria with and without S9 mix onto Petri plates of solid agar. The plates were incubated at 37°C for 48 hours, then the colonies counted using an automatic counter. The protocol corresponds to OECD TG 471 "Bacterial Reverse Mutation Test" (1997) except for the lack of a fifth E coli or TA 102 bacterial test strain.

Transfluthrin was tested in a range from 20-12,500 µg/plate on four tester strains of bacteria – TA 1535, TA 100, TA 1537, and TA 98. The doses selected within this range were based on cytotoxicity observed above 500 µg/plate. Each condition was tested on four plates, and the results given as the mean value of those plates. Positive and negative controls were run concurrently. Positive controls for the tests without metabolic activation were specific for each bacterial strain; Na-Az was used with TA 1535, NF was used with TA 100, 4-NPDA was used for TA1537 and TA 98. 2-AA was the positive control for all strains in tests with S9 metabolic activation. The negative control was 0.1 ml DMSO. No untreated negative control was used since the literature and experience of this laboratory indicates that DMSO does not have an effect on spontaneous mutation rate of these test strains.

Laboratory criteria for acceptability of the test, and for a positive or negative result, were stated.

Results and discussion

No detectable biologically relevant change in value from the respective negative control was detected. No mutagenic effects attributable to transfluthrin were seen. The positive and negative controls induced the expected range of values, demonstrating the system's sensitivity and the activity of the S9 mix.

See data summary in Table A6.6.1-01

Conclusion

Under the conditions of this assay, transfluthrin was not genotoxic.

Reliability

2

Deficiencies

Lack of one type of test strain as recommended by OECD 471. The study complied with the guideline of the day; the deficiency is not thought to markedly impair the value of the study.

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	17-4-2007
Materials and Methods	Applicants version is acceptable.
Results and discussion	Applicant's version is adopted
Conclusion	Transfluthrin is negative in the 4 bacterial strains tested, but to comply with OECD 471, a 5th bacterial strain should have been tested.
Reliability	1
Acceptability	Acceptable, except for the lack of testing the substance in the 5th bacterial test strain (i.e. E.Coli WP2 uvrA or TA 102)
Remarks	A second, non-key, gene mutation study in bacteria was submitted, confirming the negative results with and without S9-mix in the same 4 bacterial strains. No bacteriotoxicity was seen up to the highest dose tested (12500 µl/plate) but precipitation of test substance was noted in dose levels of 2500 µg/plate and higher. A non-key study in the yeast Saccharomyces cerevisiae D7 showed no mitotic recombination induced by transfluthrin in presence or absence of S9-mix.
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.6.1-1. Results of *Salmonella* Reverse Mutation Assay with Strain TA1535

Concentration [µg/plate]	Mean number of revertant colonies Strain TA1535		Mean number of revertant colonies Strain TA100		Mean number of revertant colonies Strain TA1537		Mean number of revertant colonies Strain TA98	
	— S9	+ S9	— S9	+ S9	— S9	+ S9	— S9	+ S9
0	17 12	12 13 15	108 65	148 99 100	7 5	8	14 20	34 25 32
20	16	12	114	146	5	8	15	29
100	19	16	97	134	5	9	13	29
500	14	16	107	145	5	8	14	34
775	12	18 14	64	78 122	6	6 7	10	20 26
1550	10	14 14	50	91 91	6	4 8	10	14 28
2500	18	14	115	147	6	7	14	25
3100	15	12 11	57	68 119	6	6 8	10	17 31
6200	11	12 12	33	100 140	7	6 6	9	22 27
12400	11	12 13	59	94 122	6	9 6	14	27 25
12500	18	14	123	135	7	7	13	23
Positive Controls	1010 998	503 337 504	315 474	1347 508 3055	75 76	54 56 263	80 116	1114 214 1469

Comments: positive controls: without S9-mix – Na Az (TA1535), NF (TA100), or 4-NPDA (TA 1537, TA98); with S9-mix – 2-AA (all strains).

Cytotoxic effects were seen at concentrations above 500 µg/plate.

Some tests were performed two or three times (represented by two or three values in a box). A number represents 4 plates tested simultaneously and the result averaged.

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SECTION A6.6.2

BPD Data set IIA/ Annex Point VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (human lymphocytes)

			Official use only
		77 REFERENCE	
77.1	**Reference**	[redacted] (1990).	
		In vitro cytogenetic study with human lymphocytes for the detection of induced clastogenic effects. [redacted]	
		[redacted] Report No.T 5032550 [BES Ref: MO-03-010002]	
		Report date: February 7, 1990	
		Unpublished	
77.2	**Data protection**	Yes	
77.2.1	Data owner	Bayer AG	
77.2.2			
77.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I	
		78 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes	
		The methods used are comparable to OECD 473 (July 1997)	
78.1	**GLP**	Yes	
78.2	**Deviations**	No	X
		79 MATERIALS AND METHODS	
3.1	**Test material**	NAK 4455 (transfluthrin)	
3.1.1	Lot/Batch number	Lot No. 250987	
3.1.2	Specification	As given in Section 2 of Doc IIIA	
3.1.2.1	Description	Brownish liquid of weak odor	
3.1.2.2	Purity	94.9% (average of two analyses)	
3.1.2.3	Stability	Confirmed by analysis at least for the duration of the treatment period. A stability test after mixing in the solvent (dimethylsulphoxide - DMSO) did not detect a relevant change in the active ingredient.	
3.2	**Study Type**	*In vitro* mammalian chromosome aberration test	
79.1.1	Organism/cell type	Human lymphocytes from the blood of one male and one female healthy donor were used for each trial. Blood was obtained, and the lymphocytes isolated for culture immediately.	

Doc. IIIA Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (human lymphocytes*)*

SECTION A6.6.2

BPD Data set IIA/ Annex Point VI.6.2

79.1.2	Deficiencies / Proficiencies	Not applicable
79.1.3	Metabolic activation system	S9-mix used: Animal species: Male Sprague-Dawley rats, body weight 200-300g Organ: Liver Induction: Yes, intraperitoneal injection of Aroclor 1254 in corn oil 5 days before sacrifice Composition of S-9 mix per 100 ml: S-9 fraction (50 ml, 3.0 ml ice cold KCl per 1.0 g liver before homogenization), $MgCl_2$ (271 mg), KCl (410 mg), glucose-6-phosphate disodium salt (298.5 mg), NADP disodium salt (525 mg), phosphate buffer (50 ml of 100 mM).
79.1.4	Positive control	The following substances were used: Mitomycin C (MMC) Cyclophosphamide (CP)
79.2	**Administration / Exposure; Application of test substance**	
79.2.1	Concentrations	<u>Chromosomal aberration:</u> Experiment 1: 50, 100 and 200 µg/mL in the absence of S9-mix 50, 100, and 200 µg/mL in the presence of S9 mix Experiment 2: 120, 160, and 200 µg/mL in the absence of S9-mix The concentrations used were based on a pilot study in which the concentrations were 100, 500, 1000, 5000, and 10,000 µg/mL. 200 µg/mL was chosen as the maximum concentration based on substance precipitation and reactions with the polypropylene culture materials at higher concentrations.. Concentrations of the repeat test were based on substance toxicity and gaps in the range from 120 – 200 µg/mL without the S9 mix.
79.2.2	Way of application	Solutions of transfluthrin in dimethylsulphoxide (DMSO) were applied directly to cells within the culture medium.

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Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (human lymphocytes*)*

SECTION A6.6.2

BPD Data set IIA/ Annex Point VI.6.2

79.2.3	Pre-incubation time	The cultures were incubated for 48 hours at 37°C prior to application of the test material. Duplicate cultures were set up for each test, but the duplicate was only used if the first culture had insufficient cells.	
		Experiment 1: The cultures were treated with the test material (both in presence and absence of S9-mix) for a period of 2.5 hours at 37°C. At the end of the treatment period the cells were washed in fresh medium and incubated for a further 18 hours at 37°C (recovery period) in fresh medium with S9 mix. Colcemid was added to arrest cells in metaphase (18 hours after initiation of treatment), then cells were harvested 24 hours after treatment was initiated.	X X X
		Experiment 2 (confirmatory test for dose in the absence of S9): The same treatment as experiment 1 was performed in the absence of S9-mix to address the data gap between 100 and 200 µg/mL.	
79.2.4	Other modifications		
79.3	**Examinations**		
79.3.1	Number of cells evaluated	At the end of the test schedule, cell cultures were fixed in ethanol/glacial acetic acid, then the cells were sedimented and isolated, and applied to slides. The slides were Giemsa stained, then coverslipped for light microscopy. Slides were examined at 1000x magnification under a planapochromatic lens.	
		Structural aberrations - 100 metaphase cells per culture per sex were evaluated, for a total of 200 per dose. Cells from duplicate cultures were used when needed. Polyploidy/endoreduplication – 400 cells were evaluated.	
		A test was considered positive if there was a reproducible, dose-dependent and statistically significant increase in the aberration rate. A test was considered equivocal if there was an increase which was statistically significant but not concentration-related, or if a concentration-related increase occurred which was not statistically significant.	
		80 RESULTS AND DISCUSSION	
4.1	**Genotoxicity**		
4.1.1	without metabolic activation	No	
4.1.2	with metabolic activation	No	
80.1	**Cytotoxicity**	Yes; cytotoxicity was noted during the determination of the mitotic index. Data was not provided, but the summary states that a significant concentration-related cytotoxic effect of NAK 4455 (transfluthrin) was seen on the test groups, both with and without S9 mix.	

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SECTION A6.6.2

BPD Data set IIA/ Annex Point VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (human lymphocytes*)*

		81 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	The test material is NAK 4455, common name transfluthrin. The protocol is consistent with the guidelines of the OECD, TG 473 (July 1997) and is fully acceptable to meet data requirements for a mammalian cell chromosome damage assay.	X
		The test material was dissolved in DMSO, and stability was confirmed. Human lymphocyte primary cell cultures were incubated for 48 hours at 37ºC prior to application of the test solution and the S9 fraction. The test solution was added to the cell cultures in a series of two experiments. One experiment was done at concentrations of 50, 100, and 200 µg/mL in the absence of S9-mix, and at 50, 100, and 200 µg/mL in the presence of S9 mix; and a second experiment at concentrations of 120, 160, and 200 µg/mL in the absence of S9-mix. Cultures were incubated for 2.5 hours with the test substance and S9 mixture, then washed and incubated again with culture medium containing the S9 mixture. Sixty nine hours after beginning the culture, colcemid was added to arrest metaphase cells, then the cells were fixed and prepared for examination three hours later (at 72 hours after the culture was initiated).	X
		In experiment 1 the cultures were treated with the test solution both in the presence and absence of S9-mix. On the basis of results from this test, a second experiment was performed to determine the effect of doses between 100 and 200 µg/mL in the absence of S9.	
		The negative (solvent) and positive controls were run concurrently. The positive control substances, mitomycin C (MMC) at 0.15 µg/mL and cyclophosphamide (CP) at 15 µg/mL were used to confirm the sensitivity of the test system.	
81.1	**Results and discussion**	Transfluthrin showed dose-related cytotoxicity during determination of the mitotic index, both with and without S9 mix. This limited testing concentrations to a maximum of 200 µg/mL.	
		There was no evidence of clastogenicity at concentrations up to 200 µg/mL, either with or without metabolic activation.	
		See Tables A6_6_2-1a, b below for a summary of the results.	
		Both positive control compounds, mitomycin C and cyclophosphamide, induced marked and appropriate increases in the incidence of structurally aberrant cells. Negative control values fell within the historical control range.	
81.2	**Conclusion**	Transfluthrin is not clastogenic (does not appear to induce chromosomal aberrations) in cultured mammalian cells (human lymphocytes) *in vitro* in a study which fully complies with guideline requirements.	
81.2.1	Reliability	1	
81.2.2	Deficiencies	No	X

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date

23-4-2007 and 19-1-2011 and 22-3-2011 and 2-10-2012

Materials and Methods

3.3.3. - In the culture without S9, incubation of lymfocytes with NAK 4455 was continue until sampling (24 hours), not 2.5 hours.

- It is not stated which culture medium was used for the cultured lymfocytes with S9 after washing.

- Colcemid was added 21 hours after initiation of treatment, not 18 hours.

Results and discussion

Applicant's version is adopted.

19-1-2011: Conclusion is adopted due to comments from MSs. A trend-test shows a statistically significant concentration-related increase in cells with chromosome aberrations in the metabolically activated test. In a likelihood-ratio test comparing one stage model with the no-response model, the significance level was p=0.038. Combined testing of all three trials resulted in a level of p=0.012. A trend test is appropriate for testing concentration-related changes. This would mean that the study result could be judged as positive, indicating that further testing is required.

22-3-2011: The applicant does not agree that the in-vitro cytogenic study in mammalian cells was positive as there was no dose-related increase in the experiment with metabolic activation (1, 1, 5, 5), the response was not statistically significant according to the chi^2 test, the Cochran-Armitage trend test is considered inappropriate (see position paper by [redacted]n) and all test data are clearly within the historical control range of the performing laboratory (HCD and contained in position paper by [redacted], March 2011). More detailed argumentation is presented in position paper ([redacted], March, 2011).

Conclusion	Transfluthrin does not appear to induce chromosomal aberrations in cultured human lymphocytes. TMI2011 couldnot make a conclusion on this in vitro study concerning the use of the statistical test and the historical control data. Therefore, the datapackage at the moment is not enough to conclude that the substance is not negative and also not positive. With the additional in vitro study (voluntarily agreed by the applicant) and eventually an in vivo study (if in vitro study is +) the RMS can make a definitive conclusion. So, an in vitro micronucleus study will be performed by the applicant. If it is negative the subject is sufficiently covered. If it is positive, an additional in vivo study needs to be performed (in vivo comet assay or on spleen cells or another acceptable alternative for the in vivo micronucleus test). Applicant will need to adopt the waiver for the in vivo study even if the new in vitro study is negative. The applicant will need to include more detail on the discussions about the in vivo micronucleus test and previous in vitro tests (see also 6.6.2 and 5). *2-10-2012: The applicant performed two new studies one in vitro and one in vivo micronucleustest. The in vitro study has positive results and the in vivo study has negative results.*
Reliability	2 Deviations from OECD 473: - S9-fraction 0.5%, instead of recommended 1-10% - Incubation with test substance was 24 hours in cultures without S9, and only 2.5 hours in cultures with S9, instead of 3-6 hours for both S9- and S9+ cultures in the first experiment. No duplicate cultures were used.
Acceptability	Acceptable; deviations from OECD 473 are only minor deviations.
Remarks	Lack of clastogenic activity of NAK 4455 was further established in a non-key Sister Chromatoid Exchanges (SCE)-assay, an indicator test for DNA damage. No ability of NAK 4455 was found to induce SCE's in Chinese Hamster Ovary (CHO) cells, with or without metabolic stimulation. 22-3-2011: Cytotoxicity was noted during the determination of the mitotic index. Data was not provided, but the summary states that a significant concentration related cytotoxic effect of NAK 4455 (transfluthrin) was seen on the test groups, both with and without S9 mix. In Table A6.6.2-1 the pos. control with S9 to cyclophosphamide was not mentioned.

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Section A6.6.2

BPD Data set IIA/
Annex Point VI.6.VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (human lymphocytes)

Table A6.6.2-1 Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis of Metaphase Cells

		Control DMSO solvent only	50 µg/mL	100 µg/mL	200 µg/mL	Positive control (MMC)
Metabolic activation		**-S9**	**-S9**	**-S9**	**-S9**	**-S9**
% Metaphases with exchanges (200 cells)		-	-	-	-	9.5
% Metaphases with aberrations	Evaluated metaphases	200	200	200	100	200
	Including gaps	5.0	7.5	7.5	20.0	46.5*
	Excluding gaps	0.5	1.0	2.0	2.0	34.0*
Polyploidy and endoreduplicated cells	Evaluated metaphases	400	400	400	300	400
	% polyploid	-	-	0.5	0.3	0.3
Metabolic activation		**+S9**	**+S9**	**+S9**	**+S9**	**+S9**
% Metaphases with exchanges (200 cells)		-	-	-	-	1.5
% Metaphases with aberrations	Evaluated metaphases	200	200	200	200	200
	Including gaps	9.0	10.0	11.5	9.0	39.5*
	Excluding gaps	0.5	0.5	2.5	2.5	20.0*
Polyploidy and endoreduplicated cells	Evaluated metaphases	400	400	400	400	400
	% polyploid	-	-	-	0.3	-
Comments	• $P<0.01$ in Chi^2 test; • female cultures at 200 µg/mL were not available for analysis so only 100 cells total were examined.					

Doc. IIIA/ Section A6.6.2

BPD Data set IIA/ Annex Point VI.6.VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (Chinese hamster lung cells)

Table A6.6.2-2 Table for Cytogenetic In-Vitro-Test: Chromosomal Analysis (human lymphocytes)

		Control DMSO solvent only	120 µg/mL	160 µg/mL	200 µg/mL	Positive control (MMC)
Metabolic activation		**-S9**	**-S9**	**-S9**	**-S9**	**-S9**
% Metaphases with exchanges (200 cells)		-	-	-	-	6.5*
% Metaphases with aberrations	Evaluated metaphases	200	200	200	100	200
	Including gaps	6.5	5.0	6.0	6.0	38.0*
	Excluding gaps	0.5	1.0	0.5	2.0	21.5*
Polyploidy and endoreduplicated cells	Evaluated metaphases	400	400	400	300	400
	% polyploid	-	-	0.3	-	-
Comments	• P<0.01 in Chi^2 test • female cultures at 200 µg/mL were not available for analysis so only 100 cells total were examined.					

Document IIIA — Genotoxicity in vitro

SECTION 6.6.2/02 — *In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes

Annex Point IIA VI.6.6

Official use only

		82 REFERENCE
82.1	**Reference**	[redacted] (2012) Transfluthrin: Induction of micronuclei in cultured human peripheral blood lymphocytes. [redacted] Lab Study No. 8247245; BES Ref.: M-423955-01-1 Report date: February 2012 Unpublished
82.2	**Data protection**	Yes
82.2.1	Data owner	Bayer CropScience AG
82.2.2		
82.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		83 GUIDELINES AND QUALITY ASSURANCE
83.1	**Guideline study**	based on OECD guideline 487 (2010)
83.2	**GLP**	Yes
83.3	**Deviations**	None that would prejudice the validity of the study in anyway
		84 MATERIALS AND METHODS
84.1	**Test material**	Transfluthrin
84.1.1	Lot/Batch number	Batch No. PNLO000030
84.1.2	Specification	As given in sections 2
84.1.2.1	Description	Colourless melt
84.1.2.2	Purity	97.6%. 1-R trans isomer: 99.1% area 1 S- trans isomer: 0.9% area
84.1.2.3	Stability	stored at 15-25°C protected from light
84.2	**Study Type**	Micronucleus test in vitro
84.2.1	Organism/cell type	Human Blood Lymphocytes. Blood from two healthy, non-smoking female volunteers from a panel of donors at Covance
84.2.2	Deficiencies / Proficiencies	Not applicable

Document IIIA — Genotoxicity in vitro

SECTION 6.6.2/02 — *In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes

Annex Point IIA VI.6.6

84.2.3	Metabolic activation system	The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was obtained from Molecular Toxicology Incorporated, USA where it is prepared from male Sprague Dawley rats induced with Aroclor 1254. The batches of MolTox™ S-9 were stored frozen in aliquots at <-50°C prior to use. Each batch was checked by the manufacturer for sterility, protein content, ability to convert known promutagens to bacterial mutagens and cytochrome P-450-catalyzed enzyme activities (alkoxyresorufin-O-dealkylase activities). The S-9 mix was prepared in the following way: Glucose-6-phosphate (G6P: 180 mg/mL), β-Nicotinamide adenine dinucleotide phosphate (NADP: 25 mg/mL), Potassium chloride (KCl: 150 mM) and rat liver S-9 were mixed in the ratio 1:1:1:2. For all cultures treated in the presence of S-9, an aliquot of the mix was added to each cell culture to achieve the required final concentration of test article in a total of 10 mL. The final concentration of the liver homogenate in the test system was 2%. Cultures treated in the absence of S-9 received an equivalent volume of KCl (150 mM).
84.2.4	Positive control	Mitomycin C (MMC), Cyclophosphamide (CPA), Vinblastine (VIN). For pulse treatments, MMC (without S-9) and CPA (with S-9) were used as the positive controls. For the continuous 24+0 hour without S-9 treatment, VIN was used as the positive control
84.3	**Administration / Exposure; Application of test substance**	
84.3.1	Concentrations	The test compound was dissolved in DMSO The solubility limit in culture medium was in the range of 123.5 to 247.1 µg/mL, as indicated by precipitation at the higher concentration which persisted for at least 20 hours after test article addition. A maximum concentration of 3710 µg/mL was selected for the cytotoxicity Range-Finder Experiment, in order that treatments were performed up to 10 mM, the maximum recommended concentration according to current regulatory guidelines (OECD, 2010). Concentrations for the Micronucleus Experiment were selected based on the results of this cytotoxicity Range-Finder Experiment. Cytotoxicity Range-Finder: 13.46 to 3710 µg/mL Micronucleus Experiment: 50 to 300 µg/mL (with S-9) 5 to 100 µg/mL (without S-9) **For details see Table A6.6.2/02-1**
84.3.2	Way of application	Cytotoxicity Range-Finder: Immediately prior to treatment, all 24+0 hour cultures had 0.1 mL of culture medium removed to give a final pre-treatment volume of 9.3 mL. S-9 mix or KCl (0.5 mL per culture) was added appropriately. Cultures were treated with the test article or vehicle controls (0.1 mL per culture) as indicated in **Table A6.6.2/02-2**. Positive control treatments were not

		included. Cyto-B, formulated in DMSO, was added directly (0.1 mL per culture) to all 24+0 hour cultures at the time of treatment to give a final concentration of 6 µg/mL per culture. The final culture volume was 10 mL. Cultures were incubated at 37 ± 1°C for the designated exposure time. Micronucleus Experiment: Immediately prior to treatment, all 24+0 hour cultures had 0.1 mL of culture medium removed to give a final pre-treatment volume of 9.3 mL. S-9 mix or KCl (0.5 mL per culture) was added appropriately. Cultures were treated with the test article, vehicle or positive controls (0.1 mL per culture) as indicated in **Erreur ! Source du renvoi introuvable.**. The final culture volume was 10 mL. Cyto-B, formulated in DMSO, was added directly (0.1 mL per culture) to all continuous cultures at the time of treatment to give a final concentration of 6 µg/mL per culture. Cultures were incubated at 37 ± 1°C for the designated exposure time
84.3.3	Pre-incubation time	Not applicable
84.3.4	Other modifications	Not applicable
84.4	**Examinations**	
84.4.1	Number of cells evaluated	Where possible, one thousand binucleate cells from each culture (2000 per concentration) were analysed for micronuclei. The number of cells containing micronuclei and the number of micronuclei per cell on each slide was noted.
		85 RESULTS AND DISCUSSION
85.1	**Genotoxicity**	
85.1.1	Without metabolic activation	Treatment of cells with Transfluthrin for 3+21 and 24+0 hours in the absence of a rat liver metabolic activation system (S-9) resulted in frequencies of MNBN cells that were similar to those observed in concurrent vehicle controls for all concentrations analysed. The MNBN cell frequency of all treated cultures fell within normal ranges. See **Table A6.6.2/02-3 and Table A6.6.2/02-5**
85.1.2	With metabolic activation	Treatment of cells for 3+21 hours in the presence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.01$) than those observed in concurrent vehicle controls for the highest two concentrations analysed (230 and 240 µg/mL). The MNBN cell frequencies of both treated cultures at the highest two concentrations and single cultures at the lowest two concentrations (200 and 220 µg/mL) exceeded the normal range. Although the toxicity of the highest concentration analysed (63% reduction in RI at 240 µg/mL) was higher than the target cytotoxicity range of 55 ± 5%, it was deemed acceptable for analysis. However, both cultures also exceeded the normal range at 230 µg/mL which induced 39% toxicity. A concentration-related increase in the proportion of cells with micronuclei was observed at those concentrations below 50% toxicity, indicating a positive result.

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SECTION 6.6.2/02

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Genotoxicity in vitro

***In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes**

See **Table A6.6.2/02-4**

85.2 Cytotoxicity

It may be noted that precipitate was observed in a single culture at 150 µg/mL following 3+21 hour treatment in the presence of S-9 at both the end of treatment and at harvest. It is unusual for this to occur in only one culture especially at a concentration which is approximately half the concentration at which precipitate was seen at the end of treatment elsewhere (280 µg/mL). This precipitate may be S-9 protein related and not a true representation of the test article; in any case, this concentration was not required for micronucleus analysis and was considered to not affect study interpretation

86 APPLICANT'S SUMMARY AND CONCLUSION

86.1 Materials and methods

Transfluthrin was tested in an in vitro micronucleus assay using duplicate human lymphocyte cultures prepared from the pooled blood of two female donors in a single experiment. Treatments covering a broad range of concentrations, separated by narrow intervals, were performed both in the absence and presence of metabolic activation (S-9) from Aroclor 1254 induced animals. The test article was formulated in anhydrous analytical grade dimethyl sulphoxide (DMSO) and the highest concentrations used in the Micronucleus Experiment, limited by toxicity, were determined following a preliminary cytotoxicity Range-Finder Experiment.

Treatments were conducted (as detailed in the following summary table) 48 hours following mitogen stimulation by phytohaemagglutinin (PHA). The test article concentrations for micronucleus analysis were selected by evaluating the effect of Transfluthrin on the replication index (RI). In the Micronucleus Experiment, micronuclei were analysed at three or four concentrations and a summary of the micronucleus data is presented in **Table A6.6.2/02-6**

Appropriate negative (vehicle) control cultures were included in the test system under each treatment condition. The proportion of micronucleated binucleate cells (MNBN) in these cultures fell within current historical vehicle control (normal) ranges. Mitomycin C (MMC) and Vinblastine (VIN) were employed as clastogenic and aneugenic positive control chemicals respectively in the absence of rat liver S-9. Cyclophosphamide (CPA) was employed as a clastogenic positive control chemical in the presence of rat liver S-9. Cells receiving these were sampled in the Micronucleus Experiment at 24 hours after the start of treatment; all compounds induced statistically significant increases in the proportion of cells with micronuclei.

All acceptance criteria were considered met and the study was therefore considered as valid.

Document IIIA — Genotoxicity in vitro

SECTION 6.6.2/02 — *In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes

Annex Point IIA VI.6.6

86.2	**Results and discussion**	Treatment of cells with Transfluthrin for 3+21 and 24+0 hours in the absence of a rat liver metabolic activation system (S-9) resulted in frequencies of MNBN cells that were similar to those observed in concurrent vehicle controls for all concentrations analysed. The MNBN cell frequency of all treated cultures fell within normal ranges. Treatment of cells for 3+21 hours in the presence of S-9 resulted in frequencies of MNBN cells that were significantly higher ($p \leq 0.01$) than those observed in concurrent vehicle controls for the highest two concentrations analysed (230 and 240 µg/mL). The MNBN cell frequencies of both treated cultures at the highest two concentrations and single cultures at the lowest two concentrations (200 and 220 µg/mL) exceeded the normal range. Although the cytotoxicity of the highest concentration analysed (63% reduction in RI at 240 µg/mL) was higher than the target cytotoxicity range of 55 ± 5%, it was deemed acceptable for analysis. However, both cultures also exceeded the normal range at 230 µg/mL which induced 39% cytotoxicity. A concentration-related increase in the proportion of cells with micronuclei was observed at those concentrations below 50% toxicity, indicating a positive result. Results are summarised in **Table A6.6.2/02-6**
86.3	**Conclusion**	It is concluded that Transfluthrin induced micronuclei in cultured human peripheral blood lymphocytes following 3+21 hour treatment in the presence of a rat liver metabolic activation system (S-9). Transfluthrin did not induce micronuclei in cultured human peripheral blood lymphocytes following 3+21 and 24+0 hour treatments in the absence of S-9. Treatments were performed up to cytotoxic concentrations
86.3.1	Reliability	1
86.3.2	Deficiencies	None reported

Document IIIA Genotoxicity in vitro

SECTION 6.6.2/02 *In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes

Annex Point IIA VI.6.6

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	21 September 2012
Materials and Methods	The version of the applicant is acceptable
Results and discussion	It is concluded that Transfluthrin induced micronuclei in cultured human peripheral blood lymphocytes following 3+21 hour treatment in the presence of a rat liver metabolic activation system (S-9). Transfluthrin did not induce micronuclei in cultured human peripheral blood lymphocytes following 3+21 and 24+0 hour treatments in the absence of S-9. Treatments were performed up to cytotoxic concentrations Cytoxicity was reported, however, data on mitotic index was not provided.
Conclusion	The version of the applicant is adopted.
Reliability	1
Acceptability	acceptable
Remarks	
	Comments from ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A6.6.2/02-1: Transfluthrin Concentration Ranges Tested

Experiment	Treatment	Concentration range (mg/mL)	Final concentration range (µg/mL)
Range-Finder	3+21, -S-9	1.346 to 371.0	13.46 to 3710
	3+21, +S-9	1.346 to 371.0	13.46 to 3710
	24+0, -S-9	1.346 to 371.0	13.46 to 3710
Micronucleus Experiment	3+21, -S-9	1.000 to 10.00	10.00 to 100.0
	3+21 +S-9	5.000 to 30.00	50.00 to 300.0
	24+0, -S-9	0.500 to 10.00	5.000 to 100.0

Table A6.6.2/02-2: Treatment Scheme

Treatment	S-9	Number of cultures			
		Cytotoxicity Range-Finder		Micronucleus Experiment	
		3+21*	24+0*	3+21*	24+0*
Vehicle control	-	2	2	4	4
	+	2		4	
Test article	-	1	1	2	2
	+	1		2	
Positive controls	-			2	2
	+			2	

* Hours treatment + hours recovery

Table A6.6.2/02-3: Erreur ! Source du renvoi introuvable.**, 3+21 hour treatments in the absence of S-9 / Micronucleus Experiment**

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	6	0.60	
	B	1000	10	1.00	
	Total	2000	16	0.80	-
10.00	A	1000	7	0.70	
	B	1000	5	0.50	
	Total	2000	12	0.60	NS (9)
40.00	A	1000	6	0.60	
	B	1000	5	0.50	
	Total	2000	11	0.55	NS (21)
55.00	A	1000	5	0.50	
	B	1000	7	0.70	
	Total	2000	12	0.60	NS (54)
MMC, 0.80	A	1000	71	**7.10 #**	
	B	1000	75	**7.50 #**	
	Total	2000	146	7.30	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance
NS = Not significant
# = Numbers highlighted exceed historical vehicle control range

Table A6.6.2/02-4: Erreur ! Source du renvoi introuvable.**, 3+21 hour treatments in the presence of S-9 / Micronucleus Experiment**

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	11	1.10	
	B	1000	2	0.20	
	Total	2000	13	0.65	-
200.0	A	1000	12	**1.20 #**	
	B	1000	7	0.70	
	Total	2000	19	0.95	NS (7)

220.0	A	1000	15	**1.50 #**	
	B	1000	7	0.70	
	Total	2000	22	1.10	NS (22)
230.0	A	1000	21	**2.10 #**	
	B	1000	12	**1.20 #**	
	Total	2000	33	1.65	$p \leq 0.01$ (39)
240.0	A	1000	16	**1.60 #**	
	B	1000	15	**1.50 #**	
	Total	2000	31	1.55	$p \leq 0.01$ (63)
CPA, 12.50	A	1000	40	**4.00 #**	
	B	1000	38	**3.80 #**	
	Total	2000	78	3.90	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance
NS = Not significant
# = Numbers highlighted exceed historical vehicle control range

Table A6.6.2/02-5: Erreur ! Source du renvoi introuvable.**, 24+0 hour treatments in the absence of S-9 / Micronucleus Experiment**

Treatment (µg/mL)	Replicate	Total BN Cells Scored	Total MNBN Cells Scored	Frequency of MNBN Cells/ Cells Scored (%)	Significance § (% Toxicity)
Vehicle	A	1000	3	0.30	
	B	1000	5	0.50	
	Total	2000	8	0.40	-
10.00	A	1000	3	0.30	
	B	1000	1	0.10	
	Total	2000	4	0.20	NS (8)
30.00	A	1000	8	0.80	
	B	1000	4	0.40	
	Total	2000	12	0.60	NS (28)
50.00	A	1000	4	0.40	
	B	1000	4	0.40	
	Total	2000	8	0.40	NS (55)
VIN, 0.03	A	185	29	**15.68 #**	
	B	277	37	**13.36 #**	
	Total	462	66	14.29	$p \leq 0.001$

MNBN = Micronucleated Binucleate
§ Statistical significance
NS = Not significant
# = Numbers highlighted exceed historical vehicle control range

Table A6.6.2/02-6: Micronucleus Experiment – Results summary

Treatment	Concentration (µg/mL)	Cytotoxicity (%)	Mean MNBN cell frequency (%)	Historical Control Range (%) #	Statistical significance
3+21 hour -S-9	Vehicle [a]	-	0.80	0.10-1.60	-
	10.00	9	0.60		NS
	40.00	21	0.55		NS
	55.00	54	0.60		NS
	*MMC, 0.80	ND	7.30		$p \leq 0.001$
3+21 hour +S-9	Vehicle [a]	-	0.65	0.10-1.10	-
	200.0	7	0.95		NS
	220.0	22	1.10		NS
	230.0	39	1.65		$p \leq 0.01$
	240.0	63	1.55		$p \leq 0.01$
	*CPA, 12.50	ND	3.90		$p \leq 0.001$
24+0 hour -S-9	Vehicle [a]	-	0.40	0.10-1.40	-
	10.00	8	0.20		NS
	30.00	28	0.60		NS
	50.00	55	0.40		NS
	*VIN, 0.03	ND	14.29		$p \leq 0.001$

[a] Vehicle control was DMSO
* Positive control
#95th percentile of the observed range
NS = Not significant
ND = Not determined

Doc. IIIA Genotoxicity in vitro

In-vitro gene mutation assay in mammalian cells

SECTION A6.6.3

BPD Data set IIA/ Annex Point VI.6.3

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	87 REFERENCE	
87.1	**Reference**	[redacted] (1989). Mutagenicity study for the detection of induced forward mutations in the CHO-HGPRT, [redacted] Report No. T 7030617 [BES Ref: MO-03-010377] Report date: June 28, 1989 Unpublished
87.2	**Data protection**	Yes
87.2.1	Data owner	Bayer AG
87.2.2		
87.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I
	88 GUIDELINES AND QUALITY ASSURANCE	
2.1	**Guideline study**	Yes The methods used are comparable to OECD 476 (1997)
88.1	**GLP**	Yes
88.2	**Deviations**	No
	89 MATERIALS AND METHODS	
3.1	**Test material**	NAK 4455 (transfluthrin)
3.1.1	Lot/Batch number	Lot No. 250987
3.1.2.1	Description	Brownish liquid of weak odor
3.1.2.2	Purity	94.8%
3.1.2.3	Stability	Test substance purity was verified by analysis prior to the study, and the test batch was considered sufficiently stable for the duration of the study. A stability test (duration not specified) in the solvent did not detect an indication of a relevant change in the active ingredient. Stability was approved in the vehicle in a range from 0.02 mg/ml to 1000 mg/ml.
3.2	**Study Type**	_In vitro_ mammalian cell gene mutation test.
89.1.1	Organism/cell type	Chinese hamster ovary (CHO) cell line, subclone CHO-K1-BH_4

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Genotoxicity in vitro

***In-vitro* gene mutation assay in mammalian cells**

SECTION A6.6.3

BPD Data set IIA/ Annex Point VI.6.3

89.1.2	Deficiencies / Proficiencies	Not applicable
89.1.3	Metabolic activation system	S9 mix used: Animal species: Male Sprague-Dawley rats, body weight 200-300g Organ: Liver Induction: Yes, intraperitoneal injection of Aroclor 1254 S9 fraction was purchased from Cytotest Cell Research, FRG, (lot number 110788). The S9 fraction was tested for contamination and cytotoxicity before use.
89.1.4	Positive control	The following substances were used: Ethylmethanesulfonate (EMS), 0.9 mg/ml in non-activation trials Dimethylbenzanthracene (DMBA), 20 µg/ml in S9 activation trials
89.2	**Administration / Exposure; Application of test substance**	
89.2.1	Concentrations	0.039 - 100 µg/mL of transfluthrin in DMSO was tested; vehicle concentration did not exceed 1% w/v of the cell culture medium. There was precipitation of the transfluthrin after addition of the transfluthrin/vehicle solution to the cell culture medium. The study was therefore conducted up to 100 µg/mL, the maximum concentration obtainable.
89.2.2	Way of application	Solutions of transfluthrin in dimethylsulphoxide (DMSO) were applied directly to cells within the culture medium with reduced serum content (5%). Corresponding controls were treated concurrently.
89.2.3	Pre-incubation time	None

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Genotoxicity in vitro

***In-vitro* gene mutation assay in mammalian cells**

SECTION A6.6.3

BPD Data set IIA/ Annex Point VI.6.3

89.2.4	Experimental procedure	The test material was dissolved in DMSO, and stability was confirmed. Exponentially growing CHO cells were plated in flasks at a density of $4x10^6$ cells per flask, and after attachment 16-24 hours later, the cultures were exposed to the test article for 5 hours in culture medium with reduced (5%) serum content. Cells were exposed to nine concentrations of the a.s., ranging from 0.39 µg/mL to 100 µg/mL, for five hours, either with or without S9 metabolic activation. After exposure, the cells were resuspended and replated in duplicate flasks (density $1.5x10^6$ cells/flask) and plates (200 cells in each of 3 60 mm Petri plates). Cultures treated with S9 activation mix were treated identically except for the addition of the S9 during the 5 hour treatment period. The flasks were incubated under growth conditions and subcultured at 3 and 6 days to enhance growth and expression of induced mutations. The plates were incubated for 7 days to allow colony development and determination of the cytotoxicity associated with each level of exposure to the test article. At the end of the growth period the flask cultures were reseeded in medium without hypoxanthine but containing 6-thioguanine to select the mutants. Additionally three 60 mm plates were seeded to determine the cloning efficiency.. After a 7 day incubation period the colonies were fixed, stained with Giemsa, and counted for 6-TG resistant mutant colonies. Colonies with fewer than 50 cells were excluded from the final count. The two negative controls (solvent and medium alone) and positive controls (EMS for non-activated cultures and DMBA for activated cultures) were run concurrently to confirm the sensitivity of the test system.
89.2.5	Other modifications	None
89.3	**Examinations**	
89.3.1	Number of cells evaluated	$4x10^6$ CHO cells were treated at each dose level. 8 dishes of $2x10^5$ cells/dish were used for mutant selection. 3 dishes of 200 cells/dish were tested for cloning efficiency.

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Genotoxicity in vitro

***In-vitro* gene mutation assay in mammalian cells**

SECTION A6.6.3

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89.3.2	Acceptance criteria	An assay was considered acceptable for evaluation if the following criteria are satisfied: Independent repetition with a second assay Negative control cloning efficiency of at least 50% Highest test article concentration should be cytotoxic to at least 70% of cells; lowest concentration should be non-cytotoxic Mutation frequency – background should not exceed 25 x 10^{-6} cells, experimental mutant frequency is acceptable only when cloning effeiciency is greater than 10%, each assay must determine the frequency of at least four treated cultures or a minimum of five dishes, the positive control must be at least three times the negative control and/or reproducible, and results are suspicious if a dose produces mutation at twice the rate of the negative control but not in a dose-responsive or reproducible manner.
89.3.3	Statistical analysis	A POISSON heterogeneity test was used to determine statistically significant increases in mutant frequency. Type I error rate could be adjusted if a multiplicity of tests are run.
89.3.4	Confirmatory Assay?	Yes; second trial for confirmation of results of first trial
		90 RESULTS AND DISCUSSION
4.1	**Genotoxicity**	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
90.1	**Cytotoxicity**	Cytotoxicity was noted at and above 50 µg/ml. Based on cytotoxicity, doses were selected for the gene mutation assay to range from 0 – 90% reduction in colony forming ability. The doses tested for mutagenicity ranged from 25 to 100 µg/ml.

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Genotoxicity in vitro

***In-vitro* gene mutation assay in mammalian cells**

SECTION A6.6.3

BPD Data set IIA/ Annex Point VI.6.3

91 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Exponentially growing CHO cells were treated with the test substance with and without S9 activation mix for 5 hours in a culture flask. Subcultures were made at 3 and 6 days to develop colonies and establish cytotoxicity of the dose concentration. After 7 days cells were reseeded onto culture plates without hypoxanthine and contained 6-thioguanine to select mutant cells. After growing on the plates for 7 days the colonies were fixed and stained. Positive and negative controls were run concurrently.
91.1	**Results and discussion**	At concentrations up to its limit of solubility transfluthrin showed dose-related cytotoxicity as seen by decreases in relative population growth and cloning efficiency. However, during conditions of activation with S9 only low cytotoxicity was observed. There were neither dose-related nor reproducible increases in mutant frequency compared to the negative controls after treatment with transfluthrin. However, the positive controls EMS (nonactivated) and DMBA (activated) showed a significantly elevated mutagenic effect. Controls were within the expected historical range of results. See Tables A6_6_3-1 and A6_6_3-2 below for a summary of the results. From these results transfluthrin is considered nonmutagenic in the CHO Forward Mutation Assay, both with and without metabolic activation.
91.2	**Conclusion**	From these results transfluthrin is considered nonmutagenic in the CHO Forward Mutation Assay, both with and without metabolic activation.
91.2.1	Reliability	1
91.2.2	Deficiencies	No

Doc. IIIA/ Section A6.6.3	**Genotoxicity in vitro**
BPD Data set IIA/ Annex Point VI.6.2	*In-vitro* cytogenetic assay in mammalian cells (Chinese hamster ovary cells)

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	24-4-2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	Transfluthrin is considered not mutagenic in the CHO forward mutation assay 22-3-2011: The study without S9 was performed with 4 doses all within a factor 2 apart (50, 75, 90 and 100 µg/ml), in the range where the dose is expected that will lead to a 10 to 20% (but not less than 10%) relative survival. RMS considers this acceptable.
Reliability	1
Acceptability	Acceptable
Remarks	The version of the applicant is adopted
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc. IIIA
Section A6.6.3

BPD Data set IIA/ Annex Point VI.6.2

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (CHO cells)

Table A6.6.3-1 CHO/HGPRT ASSAY - Non-activation

Test condition[a]	Survival		Relative population growth (% of control)[a]	Total mutant colonies[a]	Absolute cloning efficiency[a] ± S.D.	Mutant frequency[a] x10^6
	Mean colony No. ± S.D.	% Vehicle control.				
Negative control	104 ± 14	77.6	78.6 132.5	8 8	91.3 ± 2 57.0 ± 9	5.6 8.8
	63 ± 4	67.7	65.0 108.6	14 14	51.8 ± 2 55.8 ± 16	16.9 17.9
Vehicle control	134 ± 14	100	100 100	12 2	78.5 ± 2 47.8 ±2	9.6 3.0
	93 ± 37	100	100 100	6 C	44.8 ± 1 63.7 ± 12	8.4 C
Positive control	28 ± 2	21.0	25.6 42.6	209 136	41.8 ± 2 24.8 ± 2	312.5* 342.7*
	10 ± 5	11.1	20.8 41.5	42 175	22.8 ± 5 26.8 ± 1	115.1* 466.4*
25 µg/ml	182 ± 18	136.4	155.8 126.1	13 10	85.5 ± 8 70.2 ± 7	9.5 8.9
	88 ± 6	94	77.0 97.8	8 9	40.7 ± 2 47.2 ± 5	12.3 11.9
50 µg/ml	44 ± 4	32.9	19.3 21.4	7 8	89.8 ± 6 62.3 ± 9	4.9 8.0
	44 ± 8	46.8	28.6 68.3	37 18	64.8 ± 3 45.5 ± 6	35.7** 24.7**
75 µg/ml	9 ± 3	7.0	1.3 1.7	1 2	74.0 ± 3 88.8 ± 3	0.8 1.6
	17 ± 7	18.6	17.6 10.4	2 6	44.2 ± 10 44.5 ± 2	2.8 8.4
90 µg/ml	29 ± 1	21.9	5.4 5.3	1 1	56.5 ± 10 45.5 ± 8	1.1 1.4
	12 ± 1	12.4	9.6 7.1	2 1	57.0 ± 12 68.0 ± 5	2.2 1.5
100 µg/ml	21 ± 5	15.7	4.3 6.6	3 0	69.2 ± 3 58.5 ± 8	3.1 0
	5 ± 1	5.4	2.8 3.2	0 0	79.3 ± 2 65.0 ± 0	0 0
Comments:	[a] - 2 trials per dose, duplicate plates within each trial Positive control – 0.9 mg/ml EMS * = p<0.05 *= p<0.05; not considered biologically significant because of the lack of a dose-response C = dish lost to contamination					

Doc. IIIA
Section A6.6.3

Genotoxicity in vitro

In-vitro cytogenetic assay in mammalian cells (CHO cells)

BPD Data set IIA/ Annex Point VI.6.2

Table A6.6.3-2 CHO/HGPRT ASSAY - S9 activation

Test condition[a]	Survival		Relative population growth[a] (% of control)	Total mutant colonies[a]	Absolute cloning efficiency[a] ± S.D.	Mutant frequency[a] x10^6
	Mean colony No. ± S.D.	% Vehicle control.				
Negative control	130 ± 6	94.9	138.2 104.6	3 3	78.8 ± 5 62.0 ± 1	2.4 3.0
	114 ± 13	91.2	113.5 128.3	15 7	84.3 ± 7 59.2 ± 6	12.7 7.4
Vehicle control	137 ± 6	100	100 100	2 2	74.5 ± 1 60.8 ± 10	19. 2.7
	125 ± 5	100	100 100	15 10	74.0 ± 8 63.5 ± 14	12.7 9.8
Positive control	118 ± 24	86.3	93.3 146.8	31 24	79.5 ± 2 46.5 ± 5	24.4* 32.3*
	121 ± 29	96.8	114.0 31.9	32 28	50.5 ± 7 47.3 ± 3	45.3* 42.3*
25 µg/ml	88 ± 8	64.4	54.6 179.9	1 0	89.7 ± 8 59.5 ± 6	0.8 0
	113 ± 13	89.9	117.4 62.2	17 10	82.2 ± 2 64.5 ± 5	12.9 9.7
50 µg/ml	192 ± 9	140.2	86.6 116.7	1 5	63.0 ± 10 77.3 ± 1	0.8 5.0
	139 ± 5	110.9	170.1 74.4	21 31	64.5 ± 3 69.8 ± 5	23.3 31.7**
75 µg/ml	147 ± 14	107.8	85.9 73.1	4 1	77.3 ± 1 79.0 ± 10	4.3 0.9
	112 ± 10	89.4	139.4 81.5	14 10	84.0 ± 11 42.8 ± 12	10.4 14.6
90 µg/ml	156 ± 2	113.8	66.6 82.8	5 3	85.7 ± 11 83.0 ± 8	3.7 2.3
	146 ± 1	116.5	214.2 53.4	19 14	74.2 ± 9 102.3 ± 22	16.0 9.8
100 µg/ml	126 ± 8	92.0	46.1 60.4	10 4	88.7 ± 21 96.8 ± 1	7.0 2.6
	96 ± 9	76.9	107.6 57.3	14 20	92.2 ± 15 83.5 ± 14	9.5 17.1
Comments:	[a] - 2 trials, duplicate plates Positive control – 20 µg/ml DMBA * = p<0.05 *= p<0.05; not considered biologically significant because of the lack of a dose-response					

Doc. IIIA

Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

SECTION A6.6.4

BPD Data set IIA/
Annex Point VI.6.4

			Official use only
		1 REFERENCE	
1.1	**Reference**	[redacted] (1988). Micronucleus test on the mouse to evaluate for clastogenic effects, [redacted] [redacted] Report No. T 9027361 [BES Ref: MO-03-010004] Report date: July 18, 1988 Unpublished	
1.2	**Data protection**	Yes	
1.2.1	Data owner	Bayer CropScience	
1.2.2	Companies with letters of access		
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I	
		2 GUIDELINES AND QUALITY ASSURANCE	
2.2	**Guideline study**	Yes; OECD guideline TG 474 was followed for this micronucleus test	
2.3	**GLP**	Yes	
2.4	**Deviations**	Yes; 48 hours is suggested as the final time endpoint for bone marrow sampling, but this test also included a 72 hour timepoint for additional assurance that any genotoxicity wou ld be detected.	X
		3 MATERIALS AND METHODS	
3.1	**Test material**	NAK 4455, common name transfluthrin	
3.1.1	Lot/Batch number	130187 (mixed batch)	
3.1.2	Specification	As given in section 2 of Doc IIIA	
3.1.3	Description	Brown, crystallized solid with slight odor	
3.1.4	Purity	95.0% pure from analysis 2 June 1987	
3.1.5	Stability	The batch was examined and approved for use during the test period. A stability test in the vehicle did not detect a relevant change in the percent active ingredient. Also, doses were prepared shortly before use, and only a single dose was given, so stability is a minor concern.	
3.1.6	Maximum tolerable dose	Doses ranging from 250 to 2500 mg/kg transfluthrin were given to animals during pilot tests, and doses larger than 375 mg/kg resulted in adverse clinical signs and some mortality. Therefore 375 mg/kg was the maximum tolerable dose and was chosen for the micronucleus test.	
3.2	**Test Animals**		
3.2.1	Species	mouse	

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SECTION A6.6.4

BPD Data set IIA/ Annex Point VI.6.4

Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

3.2.2	Strain	Bor:NMRI (SPF Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Male and virgin female
3.2.5	Age/weight at study initiation	Animals were 8-12 weeks of age, and weighed between 28-41 g.
3.2.6	Number of animals per group	5m + 5f per sampling time (24, 48, 72 hours) except for controls which were sacrificed at 24 hours only.
3.2.7	Control animals	Yes
3.3	**Administration/ Exposure**	Oral
3.3.1	Number of applications	1
3.3.2	Interval between applications	Not applicable
3.3.3	Postexposure period	24, 48, 72 h after treatment
		Oral
3.3.4	Type	Gavage
3.3.5	Concentration	375 mg/kg bw
3.3.6	Vehicle	Lutrol E 400 (medicinal-grade polyethylene glycol 400)
3.3.7	Concentration in vehicle	Not stated. As calculated from the study: 375 mg/kg in 5 mL/kg = 75 mg/mL
3.3.8	Total volume applied	5 ml/kg
3.3.9	Controls	Vehicle
3.3.10	Substance used as Positive Control	Cyclophophamide in the form of Endoxan (Asta); dose was 20 mg/kg bw administered in deionized water.
3.3.11	Controls	Positive control in deionized water, negative control Lutrol E 400 only.

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SECTION A6.6.4

BPD Data set IIA/ Annex Point VI.6.4

Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

3.4 Experimental Procedures

This study was conducted in accordance with OECD guideline 474 with the exception that an additional timepoint at 72 hours following treatment was included.

Each treatment group contained ten mice, five males and five females. The treatment substance was administered once by oral gavage, then animals were sacrificed by decapitation 24, 48, and 72 hours after dosing. Controls were sacrificed only at 24 hours postdosing. The negative control was administered vehicle, Lutrol E 400, and the positive control was administered 20 mg/kg cyclophosphamide in deionized water. The transfluthrin was administered at only one dose level, 375 mg/kg, a dose that was the maximal tolerated dose as determined by a pilot test, but that included mortality of 7 of the 40 animals dosed (ten animals for each of the three time points and one set of ten animals to replace animals that died in the first round of testing).

Marrow from one intact femur was prepared from each animal. The marrow was flushed from the femur into fetal calf serum, and made into a fine suspension, which was centrifuged for five minutes at 1000 rpm. The supernatant was removed and the sediment was then mixed to homogeneity. One drop of the sediment was placed on a clean slide and smeared thinly, then the slides were dried overnight.

The smeared marrow was stained in an automated Hema-Tek stainer, destained with methanol and coverslipped. The slides were evaluated using a light microscope at about 1000x magnification. Micronuclei when present appear as stained chromatin particles in an enucleated erythrocyte. 1000 PCEs were counted for each animal, and the number of normochromatic erythrocytes and micronuclei in any erythrocyte were counted.

Mean counts from treatment and control groups were compared by Wilcoxon's non-parametric rank sum test. A variation was considered statistically significant if its error probability was below 5% and the treatment group value was higher than the negative control. A test was considered positive if there was a relevant or significant increase in the rate of micronucleated polychromatic erythrocytes at any time point in comparison to the negative control.

3.5 Examinations

3.5.1 Clinical signs

Yes; report notes that general symptoms up to 24 hours included roughened fur, lateral position, spasm, leaping spasm, twitching, shivering and dribbling. The animals' appearance after 24 hours appeared unaffected by the dose, and their eating behaviour was normal. However, 7 of the 40 animals treated with transfluthrin died from acute effects of the compound (375 mg/kg bw).

3.5.2 Tissue

bone marrow from femur for erythrocytes

Number of animals: 5 animals of each gender per treatment group

Number of cells: 1000 polychromatic erythrocytes were counted per animal, and the number of normochromatic erythrocytes per 1000 polychromatic was noted.

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BPD Data set IIA/ Annex Point VI.6.4

Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

	Time points:	24, 48, 72 h after treatment	
	Type of cells	erythrocytes in bone marrow	
	Parameters:	polychromatic/normochromatic erythrocyte ratio	
3.6 Further remarks			
	RESULTS AND DISCUSSION.		
3.7 Clinical signs	Effects as noted in 6.4.5 above. The acute effects demonstrate that the dose used was capable of producing symptoms in the nervous system. This indicates the likely penetration of the a.s. into the bone marrow.		
3.8 Bone marrow examination	No effects related to treatment. No relevant variation was seen between males and females, so they were evaluated jointly.		
	A data summary is given below in table 6.6.4-01		
3.9 Genotoxicity	No		
3.10 Other	None		
	4 APPLICANT'S SUMMARY AND CONCLUSION		
4.1 Materials and methods	Five mice of each gender were administered 375 mg/kg bw of transfluthrin for each of three time points (sacrifice at 24, 48, and 72 hrs postdosing). Controls were sacrificed at 24 hours only. Marrow was obtained from the femur, and PCEs and NCEs were stained for chromatin particles. Cell counts from treated groups were compared to controls. A test was considered positive if there was a relevant or significant increase in the rate of micronucleated polychromatic erythrocytes at any time point in comparison to the negative control.		
4.2 Results and discussion	There were no relevant variations between males and females, so they were evaluated jointly. The ratio of polychromatic to normochromatic erythrocytes was not altered by the treatment with transfluthrin. Treatment with the positive control showed a clear increase in polychromatic erythrocytes with micronuclei, confirming the validity and sensitivity of the test system. This study shows symptoms, which demonstrate systemic absorption and infer bone marrow exposure.	X	
	See summarized data in Table IIIA6.6.4-1		
4.3 Conclusion	Transfluthrin did not cause an increase in micronucleus formation, in a fully-acceptable in-vivo study.	X	
4.3.1 Reliability	1	X	
4.3.2 Deficiencies	Yes; there is no discussion regarding the likelihood that the test substance or its metabolites reach the general circulation as requested in OECD TG 474. However, the clinical signs of twitching and spasm indicate penetration of the test compound to the nervous system, which is a strong sign that the a.s. was circulated throughout the body.		

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	26-4-2007 and 22-3-2011 and 21-9-2012
Materials and Methods	2.4 Deviations from OECD 474: Only 1 dose level has been used instead of at least 3. Only 1000 instead of 2000 immature erytrocytes were analysed per animal.
Results and discussion	4.2 Micronucleted PCE's were slightly increased in treated mice compared to control mice. Too small amount of PCE's were evaluated in only one dose group to draw conclusions on genotoxicity of transfluthrin *in vivo.*
Conclusion	Other conclusions: 4.4 Study outcome is inconclusive due to deficiencies in study protocol 22-3-2011; The gentox datapackage at the moment is not enough to conclude that the substance is not negative and also not positive. With an additional in vitro study (voluntarily agreed by the applicant) and eventually an in vivo study (if in vitro study is +) the RMS can make a definitive conclusion. So, an in vitro micronucleus study will be performed by the applicant. If it is negative the subject is sufficiently covered. If it is positive, an additional in vivo study needs to be performed (in vivo comet assay or on spleen cells or another acceptable alternative for the in vitro micronucleus test). Applicant will need to adopt the waiver for the in vivo study even if the new in vitro study is negative. The applicant will need to include more detail on the discussions about the in vivo micronucleus test and previous in vitro tests (see also 6.6.2 and 5). 21-9-2012: The applicant performed two new studies an in vitro micronucleustest and an in vivo micronucleustest.
Reliability	3
Acceptability	Not acceptable. Only one dose level was tested instead of three. Per animal only 1000 instead of 2000 PCE's have been evaluated for micronuclei. The variation in the results is considerable, by which statistical testing is useless because of lack of statistical power, and no dose-response curve can be made with only one dose tested. Repeating the study is however not necessary. This study is not required because all *in vitro* studies are negative.
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.6.4-1. Micronucleus Test In Vivo

Experimental Groups N=10	**No. of evaluated polychromatic erythrocytes**	**No. of normochromatic erythrocytes per 1000 polychromatic**	Micronucleated cells per 1000	
			Normochromatic erythrocytes	**Polychromatic erythrocytes**
Negative control - vehicle	10000	1034 ± 487	0.7 ± 0.9	1.1 ± 0.7
Transfluthrin 24 hours	10000	940 ± 225	1.1 ± 1.1	1.5 ± 1.0
Transfluthrin 48 hours	10000	1351 ± 709	0.7 ± 0.8	1.4 ± 1.2
Transfluthrin 72 hours	10000	734 ± 254	0.6 ± 0.9	1.3 ± 1.1
Positive control - cyclophosphamide	10000	978 ± 278	1.4 ± 1.3	14.9 ± 7.6*
Comments:	* ≤ 0.01 in non-parametric Wilcoxon ranking test			

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BPD Data set IIA / Annex Point VI.6.4

Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

Official use only

92 REFERENCE

92.1	**Reference**	[redacted] (2012). Micronucleus Assay In Bone Marrow Cells Of The Mouse With Transfluthrin-a.i. (NAK4455) [redacted] Lab Report No. 146500 [BES Ref: M-437175-01-1] Report date: August 24, 2012 Unpublished
92.2	**Data protection**	Yes
3.2.1	Data owner	Bayer CropScience
3.2.2	Companies with letters of access	
3.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I.

93 GUIDELINES AND QUALITY ASSURANCE

93.1	**Guideline study**	OECD guideline TG 474 "Mammalian Erythrocyte Micronucleus Test", adopted July 21, 1997 "Commission Regulation (EC) No. 440/2008 B12", dated May 30, 2008 "EPA Health Effects Test Guidelines, OPPTS 870.5395, Mammalian Erythrocyte Micronucleus Test " EPA 712-C-98-226, August, 1998
93.2	**GLP**	Yes
93.3	**Deviations**	Yes; however none of these deviations were considered to affect the validity of the study.

94 MATERIALS AND METHODS

94.1	**Test material**	Transfluthrin (NAK 4455)
94.1.1	Lot/Batch number	PNLO000031
94.1.2	Specification	As given in section 2
94.1.3	Description	Colourless melt
94.1.4	Purity	98.4% pure from analysis of 2011-07-25
94.1.5	Stability	Storage conditions 10 to 30°C. Stable for at least 2 years if stored as recommended.
94.2	**Test Animals**	
94.2.1	Species	mouse
94.2.2	Strain	NMRI

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Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

94.2.3	Source	[redacted]
94.2.4	Sex	Male and female
94.2.5	Age/weight at study initiation	Animals were 8-11 weeks of age 1st main experiment: • males: mean value 37.4 g (SD ± 1.9 g) • females: mean value 28.6 g (SD ± 1.9 g) 2nd main experiment: • males: mean value 37.2 g (SD ± 2.3 g) • females: mean value 30.4 g (SD ± 2.5 g)
94.2.6	Number of animals per group	1st main experiment: Six males and six females 2nd main experiment: Six males and six females are assigned to each test group and three males and three females are assigned to each control group
94.2.7	Control animals	Yes
94.3	**Administration/ Exposure**	Oral
94.3.1	Number of applications	1
94.3.2	Interval between applications	Not applicable
94.3.3	Post exposure period	24 h and/or 48 h after treatment
94.3.4	Type	Oral Gavage
94.3.5	Concentration	Doses ranging from 375 to 2000 mg/kg transfluthrin were given to animals during pre-experiment tests. On the basis of the observed clinical signs and deaths in pre-experiments, the following doses were estimated to be suitable: • 1750, 875 and 437.5 mg/kg b.w. for females • 500, 250 and 125 mg/kg b.w. for males However, the males of the high dose group were treated with 1000 mg/kg instead of 500 mg/kg b.w. by mistake. Due to high mortality in the high dose groups (males & females) and medium dose group (females), a second main test was conducted with an additional low dose for both sexes, a medium dose for females and a high dose for both sexes (48 hours post-treatment). The males of the high dose group in the second main experiment were treated with a lower dose (250 mg/kg b.w. instead of 500 mg/kg b.w.), due to a weighing error in the first main experiment. The following dose levels of the test item were investigated in the

		mutagenicity experiment: 24 h preparation interval: • Males: 62.5, 125, and 250 mg/kg b.w. • Females: 109.38, 218.75, and 437.5 mg/kg b.w. 48 h preparation interval: • Males: 250 mg/kg b.w. • Females: 437.5 mg/kg b.w.
94.3.6	Vehicle	Polyethylene glycol 400 (PEG 400)
94.3.7	Concentration in vehicle	Not stated. As calculated from the study: 375 mg/kg in 5 mL/kg = 75 mg/mL
94.3.8	Total volume applied	10 ml/kg b.w.
94.3.9	Controls	yes
94.3.9.1	Substance used as Positive Control	Cyclophophamide (CPA) dose was 40 mg/kg bw administered in sterile water.
94.3.9.2	Positive control	PEG 400 only.
94.4	**Experimental Procedures**	This study was conducted in accordance with OECD guideline 474.
94.4.1	Preliminary study	A preliminary study (total of six pre-experiments) on acute toxicity was performed with two animals per sex and test group under identical conditions as in the mutagenicity study concerning animal strain, vehicle, route, frequency, and volume of administration. The animals were treated once orally with the test item and examined for acute toxic symptoms at intervals of approximately 1 h, 2-4 h, 6 h, 24 h, 30 h, and 48 h after administration of the test item. The maximum tolerated dose level is determined to be the dose that causes toxic reactions without having major effects on survival within 48 hours after the first administration of the test item. Three adequately spaced dose levels at intervals of factor 2 were administered
94.4.2	Main Experiments	Before treatment the animals (including the controls) were weighed and the individual volume to be administered was adjusted to the animal's body weight. The animals received the test item, the vehicle and the positive control substance once orally. Six males and six females were treated per dose group and sampling time, except the control groups of the second main experiment with three animals only. The animals of all dose groups (except positive control) were examined for acute toxic symptoms at intervals of approximately 1 h, 2 – 4 h, 6 h, 24 h and/or 48 h after treatment of the test item and the vehicle. Sampling of bone marrow was done 24 and 48 hours after treatment. The animals were sacrificed using CO_2 followed by bleeding. The femora were removed, the epiphyses were cut off and the marrow was flushed out

with foetal calf serum, using a syringe. The cell suspension was centrifuged at 1500 rpm (390 x g) for 10 minutes and the supernatant discarded. A small drop of the re-suspended cell pellet was spread on a slide. The smear was air-dried and then stained. Two slides were made from each bone marrow sample

94.5 Examinations

94.5.1 Clinical signs: General symptoms up to 48 hours, including abdominal position, ruffled fur, tremors, eyelid closure, excitement, Straub's phenomenon and death were monitored

94.5.2 Tissue: bone marrow from femur for erythrocytes

Number of animals: A minimum of 5 animals of each gender per treatment group

Number of cells: Evaluation of the slides was performed using NIKON microscopes with 100 x immersion objectives. Per animal 2000 polychromatic erythrocytes (PCE) were analysed for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and expressed in polychromatic erythrocytes per 2000 erythrocytes.

Time points: 24 and 48 h after treatment

94.6 Further remarks

Statistical methods (nonparametric Mann-Whitney test were used as an aid in evaluating the results. However, the primary point of consideration is the biological relevance of the results.

95 RESULTS AND DISCUSSION.

95.1 Clinical signs

As estimated by six pre-experiments 500 mg/kg b.w. for the males and 1750 mg/kg b.w. for the females administered once orally were suitable as highest treatment doses. However, the males of the high dose group were treated with 1000 mg/kg instead of 500 mg/kg b.w. by mistake. Due to high mortality observed after treatment with the high dose in both sexes (males no. 38, 39, 41, 42, 64 and 66 and females no. 44, 48, 67, 69 and 72) and the medium dose in females (no. 33 and 35) of the first main experiment, an additional low dose for both sexes, a medium dose for females and a high dose for both sexes (48 hours post-treatment) were included in order to fulfil the OECD guideline requirements for a valid study. Thus, a second main experiment was necessary.

In the second main experiment one female (no. 32) died after treatment with the high dose (437.5 mg/kg b.w.). Furthermore the males of the high dose group in the second main-experiment were treated with a lower dose (250 mg/kg b.w. instead of 500 mg/kg b.w.), due to a weighing error in the first main experiment.

95.2 Bone marrow examination

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that transfluthrin did not exert any cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle control there was no

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Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval and any dose level.

40 mg/kg b.w. cyclophosphamide administered once orally was used as positive control which showed a substantial increase of induced micronucleus frequency.

A data summary is given below in table 6.6.4/02-1

95.3 Genotoxicity

Under the experimental conditions reported, transfluthrin did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse.

Therefore, transfluthrin considered to be non-mutagenic in this micronucleus assay

95.4 Other

None

96 APPLICANT'S SUMMARY AND CONCLUSION

96.1 Materials and methods

Transfluthrin was dissolved in PEG 400, which was also used as vehicle control. Cyclophophamide (CPA) at a dose of 40 mg/kg bw was used as positive control. A volume of 10 mL/kg b.w. was once orally administered. 24 h and 48 h after a single administration of the test item the bone marrow cells were collected for micronuclei analysis. Controls were sacrificed at 24 hours only

At least five males and five females per test group were evaluated for the occurrence of micronuclei. Per animal 2000 polychromatic erythrocytes (PCEs) were scored for micronuclei.

To describe a cytotoxic effect due to the treatment with the test item the ratio between polychromatic and normochromatic erythrocytes was determined in the same sample and reported as the number of PCEs per 2000 erythrocytes.

As estimated by six pre-experiments 500 mg/kg b.w. for the males and 1750 mg/kg b.w. for the females administered once orally were suitable as highest treatment doses. However, the males of the high dose group were treated with 1000 mg/kg instead of 500 mg/kg b.w. by mistake. Due to high mortality observed after treatment with the high dose in both sexes (males no. 38, 39, 41, 42, 64 and 66 and females no. 44, 48, 67, 69 and 72) and the medium dose in females (no. 33 and 35) of the first main experiment, an additional low dose for both sexes, a medium dose for females and a high dose for both sexes (48 hours post-treatment) were included in order to fulfil the OECD guideline requirements for a valid study. Thus, a second main experiment was necessary.

In the second main experiment one female (no. 32) died after treatment with the high dose (437.5 mg/kg b.w.). Furthermore the males of the high dose group in the second main-experiment were treated with a lower dose (250 mg/kg b.w. instead of 500 mg/kg b.w.), due to a weighing error in the first main experiment.

The validity of the study is not affected as the tested dose of 250 mg/kg b.w. is regarded to be only slightly below the maximum tolerated dose

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Genotoxicity in vivo

In vivo mutagenicity study, micronucleus test in mice

(MTD) and the tested dose of 1000 mg/kg b.w. in the first experiment was far above the MTD due to high mortality. Furthermore, the surviving animals treated at the high dose and the surviving females treated at the medium dose in the first main experiment did not show any increase in micronucleated cells. Finally the toxicity data of the male and female animals in this study indicated no sex difference in toxicity providing evidence on the validity of the study even if the high dose of the male animals is slightly below the MTD.

The following dose levels of the test item were investigated in the mutagenicity experiment:

24 h preparation interval:

males: 62.5, 125, and 250 mg/kg b.w.

females: 109.38, 218.75, and 437.5 mg/kg b.w.

48 h preparation interval

males: 250 mg/kg b.w.

females: 437.5 mg/kg b.w..

96.2 Results and discussion

After treatment with the test item the number of PCEs was not substantially decreased as compared to the mean value of PCEs of the vehicle control thus indicating that transfluthrin did not exert any cytotoxic effects in the bone marrow.

In comparison to the corresponding vehicle control there was no biologically relevant or statistically significant enhancement in the frequency of the detected micronuclei at any preparation interval and any dose level.

40 mg/kg b.w. cyclophosphamide administered once orally was used as positive control which showed a substantial increase of induced micronucleus frequency.

See summarized data in Table IIIA6.6.4/02-1

96.3 Conclusion

Under the experimental conditions reported, transfluthrin did not induce an increase in micronuclei in bone marrow cells of the mouse.

Therefore, transfluthrin is considered to be non-mutagenic in this micronucleus assay

96.3.1 Reliability 1

96.3.2 Deficiencies None

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	28 September 2012
Materials and Methods	250 mg/kg is used as highest dose in males due to a weighing error. The arguments of the applicant about the acceptability of this dose is: "*The validity of the study is not affected as the tested dose of 250 mg/kg bw in the males is regarded to be only slightly below the maximum tolerated dose (MTD) and the tested dose of 1000 mg/kg bw (males) in the first experiment was far above the MTD due to high mortality. Furthermore, the surviving animals treated at the high dose and the surviving females treated at the medium dose in the first main experiment did not show any increase in micronucleated cells. Finally the toxicity data of the male and female animals in this study indicated no sex difference in toxicity providing evidence on the validity of the study even if the high dose of the male animals is slightly below the MTD*".
Results and discussion	Under the experimental conditions reported, transfluthrin did not induce an increase in micronuclei in bone marrow cells of the mouse.
Conclusion	Transfluthrin is considered to be non-mutagenic in this micronucleus assay
Reliability	1
Acceptability	Acceptable.
Remarks	Other arguments from RMS to accept the study are: • One female died at 437.5 mg/kg bw and the males are more sensitive then the females (the MTD for males is 3 times lower based on the preliminary test). As a consequence it can be expected that 250 mg/kg bw will give toxic reactions. • Based on the acute oral toxicity study in which 1) one male died in the 250 mg/kg bw group, 2) toxic reactions were observed in the groups (females and males) treated with 250 mg/kg and 500 mg/kg bw, 3) one male and one female died in the 500 mg/kg bw groups, RMS could accept that 250 mg kg bw is high enough in this in vivo genotoxic study
	Comments from ...
Date	Give date of comments submitted
Materials and Methods	Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state
Results and discussion	Discuss if deviating from view of rapporteur member state
Conclusion	Discuss if deviating from view of rapporteur member state
Reliability	Discuss if deviating from view of rapporteur member state
Acceptability	Discuss if deviating from view of rapporteur member state
Remarks	

Table A6.6.4/02-1. Summary of *in vivo* micronucleus test results

Gender	test group	dose mg/kg	sampling time	PCEs with micronuclei	Range (*)	PCE per 2000

		b.w.	(h)	(%) ± sd		erythrocytes
Males	Vehicle (1st main experiment)	0	24	0.092 ± 0.058	0 - 3	1316
	Vehicle (2nd main experiment)	0	24	0.100 ± 0.087	1 - 4	1212
	Test item	62.5	24	0.092 ± 0.066	1 - 4	1265
	Test item	125	24	0.117 ± 0.052	1 - 4	1248
	Test item	250	24	0.117 ± 0.103	0 - 5	1197
	Positive control (1st main experiment)	40	24	1.783 ± 0.296	29 - 45	1166
	Positive control (2nd main experiment)	40	24	1.500 ± 0.650	15 - 38	1227
	Test item	250	48	0.142 ± 0.097	1 - 6	1270
Females	Vehicle (1st main experiment)	0	24	0.150 ± 0.077	2 - 6	1263
	Vehicle (2nd main experiment)	0	24	0.117 ± 0.076	1 - 4	1200
	Test item	109.38	24	0.133 ± 0.041	2 - 4	1272
	Test item	218.75	24	0.117 ± 0.068	1 - 4	1232
	Test item	437.5	24	0.142 ± 0.092	1 - 5	1238
	Positive control (1st main experiment)	40	24	1.417 ± 0.563	13 - 42	1220
	Positive control (2nd main experiment)	40	24	2.217 ± 0.907	25 - 61	1287
	Test item	437.5	48	0.110± 0.082	1 - 5	1262

* Number of micronucleated cells per 2000 PCEs per animal

Doc III-A **SECTION 6.6.5, 6.6.6 AND 6.6.7** **BPD Data set IIA/ Annex Point III-0§**	**Second *in-vivo* mutagenicity study** **Germ cell effects** **Genotoxicity testing of metabolites**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The following studies are not considered necessary for transfluthrin: 1. A second *in-vivo* mutagenicity study, 2. A study to assess possible germ cell effects and 3. A study to assess genotoxicity of metabolites necessary. According to the technical guidance document in support of directive 98/8/EC, if *in vitro* assays are negative, further testing is only required if metabolites of concern are formed in mammals. The results from transfluthrin *in vitro* genotoxicity testing are negative for the three tests 6.6.1, 6.6.2 and 6.6.3 and no metabolite of concern is formed in mammals. Additionally, the results from transfluthrin *in vivo* genotoxicity testing are negative. Metabolites formed in mammals are assessed in all mammalian toxicity studies performed with transfluthrin. Additionally, no indication of potential human carcinogenicity has been seen in long-term studies with transfluthrin. Further tests on this compound are therefore unnecessary and unwarranted.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	26-4-2007 and 22-3-2011 and 21-9-2012	
Evaluation of applicant's justification	It is agreed with the applicant that no further data on genotoxicity are required.	
Conclusion	*Applicant's justification is acceptable.*	
Remarks	22-3-2011: The gentox datapackage at the moment (based on TM discussion) is not enough to conclude that the substance is not negative and also not positive. With an additional in vitro study (voluntarily agreed by the applicant) and eventually an in vivo study (if in vitro study is +) the RMS can make a definitive conclusion.	

Doc III-A SECTION 6.6.5, 6.6.6 AND 6.6.7 BPD Data set IIA/ Annex Point III-0§	Second *in-vivo* mutagenicity study Germ cell effects Genotoxicity testing of metabolites
	So, an in vitro micronucleus study will be performed by the applicant. If it is negative the subject is sufficiently covered. If it is positive, an additional in vivo study needs to be performed (in vivo comet assay or on spleen cells or another acceptable alternative for the in vivo micronucleus test). Applicant will need to adopt the waiver for the in vivo study even if the new in vitro study is negative. The applicant will need to include more detail on the discussions about the in vivo micronucleus test and previous in vitro tests (see also 6.6.2 and 4). 21-9-2012: The applicant performed two new studies an in vitro micronucleustest and an in vivo micronucleustest.
	-
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc III-A **Section 6.6.5, 6.6.6 and 6.6.7** **BPD Data set IIA/ Annex Point III-0§**	**Second *in-vivo* mutagenicity study** **Germ cell effects** **Genotoxicity testing of metabolites**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The following studies are not considered necessary for transfluthrin: 1. A second *in-vivo* mutagenicity study, 2. A study to assess possible germ cell effects and 3. A study to assess genotoxicity of metabolites necessary. According to the technical guidance document in support of directive 98/8/EC, if *in vitro* assays are negative, further testing is only required if metabolites of concern are formed in mammals. The results from transfluthrin *in vitro* genotoxicity testing are negative for the three tests 6.6.1, 6.6.2 and 6.6.3 and no metabolite of concern is formed in mammals. Additionally, the results from transfluthrin *in vivo* genotoxicity testing are negative. Metabolites formed in mammals are assessed in all mammalian toxicity studies performed with transfluthrin. Additionally, no indication of potential human carcinogenicity has been seen in long-term studies with transfluthrin. Further tests on this compound are therefore unnecessary and unwarranted.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	26-4-2007 and 22-3-2011 and 21-9-2012	
Evaluation of applicant's justification	It is agreed with the applicant that no further data on genotoxicity are required.	
Conclusion	The justification of the applicant is acceptable.	
Remarks	22-3-2011; The gentox datapackage at the moment (based on TM discussion) is not enough to conclude that the substance is not negative and also not positive. With an additional in vitro study (voluntarily agreed by the applicant) and eventually an in vivo study (if in vitro study is +) the RMS can make a definitive conclusion. So, an in vitro micronucleus study will be performed by the applicant. If it is	

Doc III-A Section 6.6.5, 6.6.6 and 6.6.7 BPD Data set IIA/ Annex Point III-0§	Second *in-vivo* mutagenicity study Germ cell effects Genotoxicity testing of metabolites
	negative the subject is sufficiently covered. If it is positive, an additional in vivo study needs to be performed (in vivo comet assay or on spleen cells or another acceptable alternative for the in vivo micronucleus test). Applicant will need to adopt the waiver for the in vivo study even if the new in vitro study is negative. The applicant will need to include more detail on the discussions about the in vivo micronucleus test and previous in vitro tests (see also 6.6.2 and 5). 21-9-2012: The applicant performed two new studies an in vitro micronucleustest and an in vivo micronucleustest.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc III-A **Section 6.6.5, 6.6.6 and 6.6.7** **BPD Data set IIA/ Annex Point III-0§**	**Second *in-vivo* mutagenicity study** **Germ cell effects** **Genotoxicity testing of metabolites**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	The following studies are not considered necessary for transfluthrin: 1. A second *in-vivo* mutagenicity study, 2. A study to assess possible germ cell effects and 3. A study to assess genotoxicity of metabolites necessary. According to the technical guidance document in support of directive 98/8/EC, if *in vitro* assays are negative, further testing is only required if metabolites of concern are formed in mammals. The results from transfluthrin *in vitro* genotoxicity testing are negative for the three tests 6.6.1, 6.6.2 and 6.6.3 and no metabolite of concern is formed in mammals. Additionally, the results from transfluthrin *in vivo* genotoxicity testing are negative. Metabolites formed in mammals are assessed in all mammalian toxicity studies performed with transfluthrin. Additionally, no indication of potential human carcinogenicity has been seen in long-term studies with transfluthrin. Further tests on this compound are therefore unnecessary and unwarranted.	
Undertaking of intended data submission [ ]	Not applicable	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	26-4-2007 and 22-3-2011 and 21-9-2012	
Evaluation of applicant's justification	It is agreed with the applicant that no further data on genotoxicity are required.	
Conclusion	The justification of the applicant is acceptable.	
Remarks	22-3-2011; The gentox datapackage at the moment (based on TM discussion) is not enough to conclude that the substance is not negative and also not positive. With an additional in vitro study (voluntarily agreed by the applicant) and eventually an in vivo study (if in vitro study is +) the RMS can make a definitive conclusion. So, an in vitro micronucleus study will be performed by the applicant. If it is negative the subject is sufficiently covered. If it is positive, an additional in vivo	

Doc III-A Section 6.6.5, 6.6.6 and 6.6.7 BPD Data set IIA/ Annex Point III-0§	Second *in-vivo* mutagenicity study Germ cell effects Genotoxicity testing of metabolites
	study needs to be performed (in vivo comet assay or on spleen cells or another acceptable alternative for the in vivo micronucleus test). Applicant will need to adopt the waiver for the in vivo study even if the new in vitro study is negative. The applicant will need to include more detail on the discussions about the in vivo micronucleus test and previous in vitro tests (see also 6.6.2 and 5). 21-9-2012: The applicant performed two new studies an in vitro micronucleustest and an in vivo micronucleustest.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Chronic Toxicity / Carcinogenicity

2-year oral rat study

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	97 REFERENCE	
97.1	**Reference**	[redacted] (1993). NAK 4455. Study for chronic toxicity and carcinogenicity in Wistar rats (Administration in diet for 2 years). [redacted] [redacted] Report No. 8025696 [BES Ref: MO-03-009856] Report date: July 7, 1993. Unpublished.
97.2	**Data protection**	Yes
97.2.1	Data owner	Bayer CropScience
97.2.2	Companies with letters of access	
97.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
	98 GUIDELINES AND QUALITY ASSURANCE	
98.1	**Guideline study**	Yes OECD 453 (1981) US EPA FIFRA § 83-5 (1984)
98.2	**GLP**	Yes
98.3	**Deviations**	No
	99 MATERIALS AND METHODS	
99.1	**Test material**	NAK 4455 (transfluthrin)
99.1.1	Lot/Batch number	Mixed batch no: 130187, from 10.11.87: 250987
99.1.2	Specification	As given in sections 2 and 3
99.1.2.1	Description	Brown-yellow clear liquid after heating to 50ºC
99.1.2.2	Purity	95.0% (130187), 94.5% (250987)
99.1.2.3	Stability	Test compound content in the administered formulation was verified at the start of study, and approximately every 3 months thereafter. Stability and homogeneity were verified before beginning of study. Purity of 100% was assumed for the technical test compound. Food mixes contained 1% peanut oil to minimize dust generation.
99.2	**Test Animals**	

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99.2.1	Species	Rat
99.2.2	Strain	Wistar; Bor:WISW (SPF-Cpb)
99.2.3	Source	[redacted]
99.2.4	Sex	Male and female
99.2.5	Age/weight at study initiation	4-6 weeks Weight range at start of study 54-78 g (males) and 52-73 g (females)
99.2.6	Number of animals per group	70 rats/sex/group
99.2.6.1	at interim sacrifice	10 animals/group/sex at 12 months
99.2.6.2	at terminal sacrifice	60 animals/group/sex
99.2.7	Control animals	Yes
99.3	**Administration/ Exposure**	Oral (dietary)
99.3.1	Duration of treatment	25 months
99.3.2	Interim sacrifice(s)	After 12 months
99.3.3	Final sacrifice	After 25 months
99.3.4	Frequency of exposure	Daily (continuous in diet)
99.3.5	Postexposure period	None
		Oral
99.3.6	Type	In food
99.3.7	Concentration	Food 0, 20, 200, 2000 ppm, equivalent to: Males: 0, 1.0, 9.9, 100.4 mg/kg bw-day Females: 0, 1.4, 13.6, 142.1 mg/kg bw-day Food consumption per day ..... ad libitum
99.3.8	Vehicle	Moistened with peanut oil/ mixed into food (1% final concentration)
99.3.9	Concentration in vehicle	N/A
99.3.10	Total volume applied	Not applicable
99.3.11	Controls	Diet with peanut oil

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99.4	**Examinations**		
99.4.1	Body weight	Yes, before administration of first dose and then weekly.	
99.4.2	Food consumption	Yes, measured weekly.	
99.4.3	Water consumption	Yes, measured weekly.	
99.4.4	Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.	
99.4.5	Macroscopic investigations	Location and progression of palpable masses, skin tumours were recorded	
99.4.6	Ophthalmoscopic examination	Yes, at start of study and after 12 and 24 months for 10 male and 10 female animals in 0 and 2000 ppm groups—surroundings of the eyes and the anterior eye sections were examined for alterations, pupil reflex test was made in a darkened room, transparent eye media and eye fundus were examined after pupil dilation.	
99.4.7	Haematology	Yes	For determination of glucose, blood samples were taken in the morning from unfasted, unanaesthetised animals from one of the caudal veins. Blood samples for other parameters were taken in the morning from the retro-orbital venous plexus (under anaesthesia).
		Number of animals:	10 or 20 animals/sex/group
		Time points:	After 6, 12, 18, 24 months of treatment
		Parameters:	Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, thrombocyte count, thromboplastin time, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular cell volume (MCV), erythrocyte morphology
			Other:
99.4.8	Clinical Chemistry	Yes	
		Number of animals:	10 or 20 animals/sex/group
		Time points:	After 6, 12, 18, 24 months of treatment
		Parameters:	Sodium, potassium, phosphate, calcium, chloride, glucose, total cholesterol, urea, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, triglycerides
		Other	
99.4.9	Urinalysis	Yes	

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	Number of animals:	10 or 20 animals/sex/group
	Time points:	After 6, 12, 18, 24 months of treatment (a few days before blood sampling after approx. 16-hr fast (water ad lib.). Some parameters only measured at end of study, they are marked with a * below.
	Parameters:	Appearance, volume, osmolality*, specific gravity, pH, protein, glucose, blood, bilirubin, ketone bodies, urobilinogen, sediment (leukocytes, erythrocytes, epithelia, cylinders (protein casts) and others, e.g. bacteria, crystals), creatinine*, urea*, phosphate*, calcium*, potassium*, sodium*, chloride*
	Other	
99.4.10 Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at interim and final sacrifice	
99.4.10.1 Organ Weights	Yes	
	From:	All animals at interim or final sacrifice
	Organs:	Liver, kidneys, adrenals, testes, spleen, brain, heart, and lungs
	Other	
99.4.11 Histopathology	Yes	
	From:	All dose groups
	From:	All animals
		At interim sacrifice
		At terminal sacrifice
	Organs:	Fixed in 10% buffered formaldehyde solution (urinary bladder and lungs fixed by instillation of formaldehyde solution): adrenal glands, aorta, brain (cerebrum, cerebellum, brain stem), epididymides, oesophagus, eyes (including lids and optic nerves), femur, Harderian glands, "head" (nasal and oropharyngeal cavity), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum; remaining intestinal tissue), kidneys, lachrymal glands (extraorbital), larynx, liver, lungs, mandibular lymph node, mesenteric lymph node, ovaries (including oviducts), parathyroid glands, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, skin/mammary region, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, ureter,

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		urethra, urinary bladder, uterus, vagina and any other tissue showing changes.
		Other
99.4.12	Other examinations	Enzyme induction: At sacrifice (interim and final) 5 animals/sex/group were examined for enzyme induction in the liver, specifically: N-demethylase, O-demethylase, cytochrome P450 and carnitine acyl transferase (CAT).
		Fluoride content: At sacrifice (interim and final), the teeth and bones of 5 animals/sex/group were analysed for fluoride levels.
99.5	**Statistics**	Arithmetic group means and standard deviation were calculated for all quantitative results (except fluoride data). Test collective data were compared with control collective data using either Mann and Whitney or Wilcoxon's U test. Differences were considered significant at the 5% and 1% probability level. Data from the fluoride analysis were evaluated using Dunnett's test after one-factor analysis of variance. Comparison of survival curves used Wilcoxon's generalized test (Breslow test), a weighting proportional to respective group sized per event time.
99.6	**Further remarks**	
		100 RESULTS AND DISCUSSION
100.1	**Body weight**	Male animals in the high and mid dose groups were slightly but significantly heavier than control animals intermittently throughout the study, although the effect appeared most frequent between weeks 33 and 90. Female animals in the high dose group had a slight but significant reduction in weight intermittently throughout the study.
100.2	**Food consumption**	No treatment related effects were seen.
100.3	**Water consumption**	No treatment related effects were seen on female animals. Males in the high dose group had a slight but significantly increased water intake.
100.4	**Clinical signs**	No treatment related effects were seen
100.5	**Macroscopic investigations**	No treatment related effects were seen.
100.6	**Ophthalmoscopic examination**	No treatment related effects were seen
100.7	**Haematology**	A number of miscellaneous statistically significant effects occurred which appear to have little toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, haemoglobin levels tended to be minimally reduced in high dose males and females, haematocrit was reduced in high dose males, and mean cell haemoglobin was reduced in all treated males, throughout the study.
100.8	**Clinical Chemistry**	A number of miscellaneous statistically significant effects occurred which appear to have little toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, triglyceride levels

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		appear to be reduced in all treated males and in high dose group females. The absence of clear dose-response or of corroborative change in other parameters, suggests that the change may in part be due to fortuitously higher values in controls.
100.9	**Urinalysis**	A number of miscellaneous statistically significant effects occurred which appear to have little toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. The only consistent effect appeared to be slightly but significantly reduced density of urine in all treated males and mid and top-dose females at the 6 month time point.
100.10	**Pathology**	Interim autopsy: No treatment related effects were found up to and including the 200 ppm dose group. Seven of ten males in the 2000 ppm dose group were found to have rough kidney surfaces. Final autopsy: Liver changes (swollen, thickened, enlarged and/or presence of nodules) were noted in a few males in each treatment group and in females in the 200 and 2000 ppm dose groups. Additionally, 2 females in the 2000 ppm dose group were found to have urinary bladder nodules.
100.11	**Organ Weights**	Absolute and relative kidney and liver weights were increased in males and females in the high dose groups. At the 12-month interim autopsy, absolute kidney weight in females in the 200 ppm group was elevated. At the 24-month final autopsy absolute kidney weight was also increased in males and females in the 200 ppm dose group, at was relative kidney weight in males in the 200 ppm group and relative liver weight in all treated females.
100.12	**Histopathology**	Interim autopsy: Glomerulonephrosis was seen in males in the 200 and 2000 ppm dose groups, yellow-brown pigment deposits were seen in the tubular epithelial cells and interstitial tissue of the kidneys of both male and female animals in the 200 and 2000 ppm dose groups in an apparently dose-dependent manner. Males in the high dose group had an increased incidence of cuboid cells in the follicular epithelium of the thyroid. Final autopsy: Glomerulonephrosis was increased in males in the 200 and 2000 ppm dose groups and in females in the 20 and 200 ppm dose groups. Pigment deposition was increased in males and females in the 200 and 2000 ppm dose groups. An increased incidence of urothelial hyperplasia of urinary bladder was seen in high dose group animals, as was a slightly increased rate of thyroid hyperplasia.
100.13	**Other examinations**	Enzyme induction: O-demethylase was higher in male and female animals in the high dose group at the 12 month but not 24 month sacrifice. Cytochrome P450 was higher in all female treatment groups at 12 but not 24 months, and in high dose males at 12 but not 24 months. Carnitine acyl transferase was higher in high dose group females at 12 and 24 months. Fluoride incorporation: Fluoride levels in bones and teeth of male and female animals were statistically significantly increased in the 200 and 2000 ppm groups at both 12 and 24 months.

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100.14	**Time to tumours**	No treatment related effects were seen.
100.15	**Other**	Neoplastic lesions: No treatment related neoplastic lesions were seen at the interim autopsy. At the 24-month final autopsy a miscellany of benign and malignant tumours were seen an all groups (including controls) and were clearly not treatment related (lack of dose response, increased incidence in controls, single instance in middle dose group, etc). None of the tumours showed statistical significance for trend based on combined prevalence and death rate method of Peto. X Two or 3 hepatocellular adenomas (benign) were seen in each of the male treatment groups and not in the controls or female animals. This response was within the parameters of historical incidence of this tumour. In the adrenal glands, there was an increased incidence of medullary tumours (benign) in the male treatment groups. In the female high dose group, there was an increased incidence in mammary adenoma (benign), two lipomatous tumours (1 malignant and 1 benign) were observed in the kidneys of high dose group males, Both sexes exhibited an increased incidence of hyperplasia and also tumours (1 or 2 papilloma and carcinoma) of the urinary bladder after the administration 2000 ppm of the test substance. The tumour frequency was above historical control data.

101 APPLICANT'S SUMMARY AND CONCLUSION

101.1	**Materials and methods**	Groups of 70 male and female Bor: WISW (SPF-Cpb) rats were given NAK 4455 in the diet at concentrations of 0, 20, 200 and 2000 ppm for 12 months at which point 10 rats/sex/group were sacrificed (interim autopsy). The remaining 60 rats/sex/group were given NAK 4455 in the diet for an additional 12 months before sacrifice. Haematology, clinical chemistry, urinalysis, liver enzyme induction, measurement of fluoride levels, and gross and histopathology were performed on all animals at or just before sacrifice. Additionally, haematology, clinical chemistry and urinalysis were performed at 6, 12, 18 and 24 months. This study fulfils the requirements of OECD 453 (1981) and US EPA FIFRA § 83-5 (1984).
101.2	**Results and discussion**	No treatment induced changes in behaviour, appearance, mortality, , food or compound intake was observed. No treatment related damage to the eye was observed. The results from the haematological and clinical chemistry studies combined with histopathology, urinalysis and enzyme induction suggest that liver and kidney damage occur in both sexes exposed to 2000 ppm and likely begins at 200 ppm. In the higher dose groups, liver weights were increased, liver enzymes were induced and enlarged liver was observed. Additionally, triglyceride levels were decreased. In the treated male groups, benign hepatocellular adenomas were seen. These were within the limits of historical controls and were not statistically significant for trend, but would be consistent with a non-genotoxic mechanism of carcinogenicity, i.e. tumours subsequent to cell death and proliferation.

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Also in the higher dose groups, increased kidney weights, decreased urine density (at 6 months), increased water consumption (males only) were observed. Rough kidney surfaces were noted in high dose group males, and glomerulonephrosis and pigment deposits within the kidneys were seen in 200 and 2000 ppm dose groups. Two lipomatous tumours were observed in the kidneys of high dose group males, but these are not statistically significant and do not demonstrate a dose-response. Increased incidence of urothelial hyperplasia of urinary bladder was seen in high dose group animals. A slight, non-significant increase of (urothelial) tumours was seen in the urinary bladder of high-dose animals. It seems likely that both kidney and urinary bladder tumours are secondary to cell damage and cell proliferation.

A dose dependent increase in fluoride content in teeth and bones was seen starting at 20 ppm; the increase became statistically significant at 200 ppm.

Incidence of thyroid hyperplasia was slightly increased in high dose group animals. This may be a secondary result of altered liver physiology.

The lowest adverse effect level in this study is 200 ppm (equivalent to approximately 9.9 and 13.6 mg/kg bw-day for males and females respectively) based on liver and kidney damage in both sexes. The no observable adverse effect level is 20 ppm (equivalent to approximately 1.0 and 1.4 mg/kg bw-day for males and females respectively).

101.3 Conclusion

101.3.1 Reliability 1

101.3.2 Deficiencies During a single spot-check analysis of the homogeneity of the compound in the feed (approximately 12 weeks after start of study), it was found that the container labelled 2000 ppm contained 200 ppm feed and vice versa. The researchers were unable to determine if the containers had simply been mislabelled or if the rats had been feed the incorrect dose for their group. Even if the rats had been feed the incorrect dose, a single instance over 104 weeks should not have any significant effect on the cumulative dose received, nor should it have had an effect on blood parameters, as the first blood sample was taken 6 months after beginning of study. This deficiency is not expected to have any effect on the study.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7 March 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	4.15 The applicant states that found tumours were clearly not related to treatment. This viewpoint is not endorsed by the RMS. Urinary bladder tumours (papilloma and carcinoma), observed at 2000 ppm, are considered to be treatment-related. Otherwise, the version of the applicant is adopted
Conclusion	The applicant does not come to a conclusion on carcinogenicity The urinary bladder urothelial hyperplasia, thyroid follicular hyperplasia and increased cuboidal cells (m+f) and urinary bladder tumours (papilloma and carcinoma), observed at 2000 ppm (equal to 100.4 mg/kg bw/day), are considered to be treatment-related. The tumours in thyroid and liver are considered not related to treatment 12-07-2010 Based on new data the conclusion is adjusted, see discussion in Doc IIA.
Reliability	1
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.7(01) -1. Table for Haematology and Clinical Chemistry

Affected		0 ppm				20 ppm				200 ppm				2000 ppm			
Parameter, unit, sex		Months after start of treatment															
Haematology		6	12	18	24	6	12	18	24	6	12	18	24	6	12	18	24
Hb, g/L	M	160	161	161	160	153	157	158	158	154	156	158	154	154	152**	153**	153
	F	153	151	150	154	149**	149	148	152	146*	147	149	145**	146**	147	144*	147
HCT, L/L	M	0.482	0.493	0.507	0.501	0.470	0.479	0.503	0.493	0.475	0.479	0.503	0.487	0.474	0.472*	0.485**	0.487
	F	0.458	0.461	0.467	0.476	0.447	0.455	0.467	0.477	0.444	0.451	0.467	0.457	0.437**	0.450	0.458	0.461
MCH, pg	M	18.5	17.5	18.0	18.2	17.7**	16.9*	17.1*	17.3	17.6**	16.8*	17.1*	17.2*	17.5*	16.7	17.2*	17.1*
	F	19.0	17.9	18.4	18.8	18.7	17.8	19.0	18.3	18.3	17.5	18.0	18.2	18.8	17.8	18.3	18.4
Thrombocytes, 10^9/L	M	1018	1092	1001	971	979	1057	994	999	1001	1036	989	1042	890	1002	900	934
	F	889	999	862	791	826	970	886	872	911	961	846	827	944	1054	925	910*
Clinical Chemistry																	
ASAT, U/L	M	32.8	29.9	35.5	32.1	37.4	35.6*	36.7	35.9	34.1	31.6	33.5	40.8	38.6	30.9	36.5	34.0
	F	34.0	71.9	82.3	61.2	40.2	41.9	61.2	54.4	31.2*	40.0	89.1	77.6	30.9**	33.8*	93.8	75.5
Triglycerides, Mm	M	2.10	3.04	2.78	2.84	1.25**	1.69*	2.08*	2.27	1.24**	1.61*	2.38	2.32	1.08**	0.93**	1.49**	1.66**
	F	1.63	1.75	1.39	1.75	1.15*	1.64	1.50	1.72	1.28	1.40	1.46	1.65	0.82**	0.91**	1.02*	1.2
Months		12		24		12		24		12		24		12		24	
N-Dem, mU/g	M	143.0		83.0		125.5		83.0		114.8		80.5		175.4		89.7	
	F	81.8		70.7		73.2		70.6		66.3*		56.7*		90.0		62.0	
O-Dem, U/g	M	12.5		13.0		11.8		11.3		12.4		11.0		17.6**		13.2	
	F	11.6		10.1		12.5		11.1		13.0		10.6		16.5**		11.6	
P450, nmol/g	M	32.0		38.3		30.6		30.9**		30.3		35.2		49.6**		30.0*	
	F	29.8		34.6		39.2*		38.6		35.6*		42.6		52.9**		38.5	
CAT, U/g	M	0.52		0.56		0.39**		0.55		0.45		0.46		0.61		0.83	
	F	1.24		1.86		1.21		2.03		1.56		1.92		2.86**		2.65*	
Fluoride, mg/g ash bones	M	0.459		0.662		0.514		0.777		0.756*		1.337*		2.243*		2.814*	
	F	0.548		0.894		0.637		0.848		1.249*		1.485*		2.949*		2.793*	
Fluoride, mg/g ash teeth	M	0.108		0.133		0.121		0.120		0.242*		0.290*		0.647*		0.677*	
	F	0.138		0.221		0.142		0.164		0.267*		0.287		0.840*		0.681*	

* p <0.05, ** p,0.01, Hb = haemoglobin, HCT = haematocrit, MCH = mean cell haemoglobin, ASAT = aspartate aminotransferase, N-dem = N-demethylase, O-dem = O-demethylase, CAT = carnitine acyl transferase

Table A6.7(01) -2. Results from carcinogenicity study

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	59	59	60	60	59	60	58	60		
Mortality	2	1	3	8	8	6	4	5	-	-
Clinical signs	-	-	-	-	-	-	-	-		
Body weight	-	-	-	-	↑*	-	↑*	↓*		
Food consumption	-	-	-	-	-	-	-	-		
Overall tumour incidence (%):	44	64	55	72	56	53	62	50		
No. of animals with neoplasms	26/59	38/59	33/60	43/60	33/59	32/60	36/58	30/60	+	-
No. of animals with benign neoplasms	23/59	38/59	22/60	34/60	25/59	33/60	30/58	30/60	+	-
No. of animals with malignant neoplasms	2/59	3/59	4/60	4/60	3/59	3/60	2/58	5/60	-	-
No. of animals with > 1 neoplasm	1/59	3/59	3/60	7/60	0/59	2/60	0/58	2/60	-	-
Liver										
Hepatocellular adenoma	0/59	0/59	3/60	0/60	2/59	0/60	3/58	0/60	-	-
Carcinoma	1/59	0/59	0/60	0/60	0/59	0/60	0/58	0/60	-	-
Non-neoplastic changes										
Swollen/thickened/ enlarged	0/59	0/59	4/60	0/60	0/59	2/59	5/58	3/60	-	-
Nodule	0/59	0/59	3/60	0/60	2/59	0/60	3/58	0/60	-	-
Absolute weight (interim 12 month)	-	-	-	-	-	-	↑**	↑**		
Absolute weight (final 24 month)	-	-	-	-	-	-	↑	↑*		
Kidney										
Tumour (lipomatous)	0/59	0/59	0/60	1/60	0/59	0/60	2/58	0/60		
Carcinoma	0/59	0/59	1/60	0/60	0/59	0/60	0/58	0/60		
Non-neoplastic changes										
Glomerulonephrosis	45/59	11/59	47/60	18/60	53/59	21/60	56/58	13/60		
Pigment deposition	41/59	33/59	41/60	40/60	53/59	54/60	58/58	59/60		
Absolute weight (interim 12 month)	-	-	-	-	↑	↑*	↑*	↑*		
Absolute weight (final 24 month)	-	-	-	-	↑**	↑*	↑**	↑		

Continued

Table A6.7(01) -2. continued

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Urinary bladder										
Papilloma	0/58	0/59	0/59	0/60	0/58	0/60	2/57	1/60		
Carcinoma	0/58	0/59	0/59	0/60	0/58	0/60	1/57	2/60		
Non-neoplastic changes										
Hyperplasia	2/59	0/59	1/60	1/60	2/59	2/60	7/58	10/60	+	+
Thyroid										
C-cell adenoma	2/58	3/59	2/60	5/60	1/59	2/60	2/58	2/59	-	-
Follicular adenoma	3/58	1/59	1/60	0/60	1/59	1/60	2/58	1/59	-	-
Follicular adenocarcinoma	0/58	0/59	0/60	1/60	0/59	0/60	1/58	0/59	-	-
Non-neoplastic changes										
Follicular hyperplasia	0/59	0/59	0/60	0/60	3/59	1/60	4/58	2/60	-	-
Increased cuboidal cells	1/10	1/10	2/10	2/10	2/10	1/10	7/10	2/10	-	-

*p <0.05, ** p < 0.01,- Not significantly different than control.

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BPD Data set IIA/
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Official use only

102 REFERENCE

102.1	**Reference**	[redacted] (1993, amended 1999) NAK 4455. Study for oncogenicity in B6C3F1 mice after administration in diet for 2 years. [redacted] [redacted] Report No. T 9027514 [BES Ref: MO-03-010149] Report date: December 13, 1993 (amendment dated 07 January 1999). Unpublished
102.2	**Data protection**	Yes
102.2.1	Data owner	Bayer CropScience
102.2.2	Companies with letters of access	
102.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

103 GUIDELINES AND QUALITY ASSURANCE

103.1	**Guideline study**	Yes OECD 451 (1981) US EPA FIFRA § 82-2 (1984)
103.2	**GLP**	Yes
103.3	**Deviations**	No

104 MATERIALS AND METHODS

104.1	**Test material**	NAK 4455 (transfluthrin)
104.1.1	Lot/Batch number	Mixed batch no: 250987
104.1.2	Specification	As given in sections 2 and 3

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104.1.2.1	Description	Dark brown	
104.1.2.2	Purity	94.5- 95%	X
104.1.2.3	Stability	Test substance was stored at room temperature in laboratory cabinet and kept stable throughout the study—Stable to May 1990. Test substance was added to the powdered food in accordance with the dose plan for each successive week. Test compound content in the administered formulation was checked at the start of study, and approximately every 3 months thereafter. Stability and homogeneity were tested before beginning of study. Purity of 100% was assumed for the technical test compound, which contained 1% peanut oil to minimize dust generation. The test compound was found to be stable in the diet over 10 days within a tolerance range of 20%, it was found to be homogenous in the diet within a tolerance of 10%. The mean concentration was within 10% of the nominal concentration.	
104.2	**Test Animals**		
104.2.1	Species	Mice	
104.2.2	Strain	B6C3F1	
104.2.3	Source	[redacted]	
104.2.4	Sex	Male and female	
104.2.5	Age/weight at study initiation	5-6 weeks Weight range at start of study 18-24 g (males) and 15-20 g (females)	
104.2.6	Number of animals per group	60 mice/sex/group (+ extra 10 mice/sex/group for 0 and 1000 ppm groups)	
104.2.6.1	at interim sacrifice	10 animals/sex/group at 12 months	
104.2.6.2	at terminal sacrifice	50 animals/sex/group	
104.2.7	Control animals	Yes	
104.3	**Administration/ Exposure**	Oral	
104.3.1	Duration of treatment	24 months	
104.3.2	Interim sacrifice(s)	After 12 months	
104.3.3	Final sacrifice	After 24 months	
104.3.4	Frequency of exposure	Daily	
104.3.5	Postexposure period	None	
		Oral	

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104.3.6	Type	In food
104.3.7	Concentration	Food 0, 10, 100, 1000 ppm, equivalent to: Males: 0, 2.1, 19.7, 199.5 mg/kg bw-day Females: 0, 3.1, 33.3, 279.0 mg/kg bw-day Food consumption per day ..... ad libitum
104.3.8	Vehicle	Moistened with peanut oil/ mixed into food
104.3.9	Concentration in vehicle	Not applicable
104.3.10	Total volume applied	Not applicable
104.3.11	Controls	Diet with peanut oil
104.4	**Examinations**	
104.4.1	Body weight	Yes, before administration of first dose and then weekly.
104.4.2	Food consumption	Yes, measured weekly (based on extra 10 animals).
104.4.3	Water consumption	Yes, measured weekly.
104.4.4	Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
104.4.5	Macroscopic investigations	Palpable masses, skin tumours
104.4.6	Ophthalmoscopic examination	No.
104.4.7	Haematology	Yes Number of animals: 10 animals/sex/group Time points: After 3 (extra groups only) 12, 18 (only differential blood count), 24 months of treatment Parameters: Haematocrit, haemoglobin concentration, erythrocyte count, total and differential leukocyte count, thrombocyte count, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular cell volume (MCV) Other:
104.4.8	Clinical Chemistry	Yes

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	Number of animals:	10 animals/sex/group	
	Time points:	After 3 (extra and main group animals), 12, 24 months of treatment	
	Parameters:	Glucose, total cholesterol, urea, total bilirubin, creatinine, total protein and albumin, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase	
	Other		
104.4.9 Urinalysis	No		
104.4.10 Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at 3-month, interim and final sacrifice		
104.4.10.1 Organ Weights	Yes		
	From:	All animals at 3-month, interim or final sacrifice	
	Organs:	Liver, kidneys, testes, spleen, brain, heart, ovaries, and lungs	
	Other		
104.4.11 Histopathology	Yes		
	From:	All dose groups	X
	From:	All sacrificed animals	
	Organs:	Fixed in 10% buffered formaldehyde solution (urinary bladder and lungs fixed by instillation): adrenal glands, aorta, bone marrow (femur and sternum), brain (cerebrum, cerebellum, brain stem), cymbal gland, ears (tattooed), epididymides, oesophagus, eyes (including lids and optic nerves), femur with knee joint, gall bladder, Harderian glands, "head" (nasal and oropharyngeal cavity), heart, intestine (duodenum, jejunum, ileum, cecum, colon, rectum; remaining intestinal tissue), kidneys, lachrymal glands (extraorbital), larynx, liver, lungs, mammary gland, mandibular lymph node, mesenteric lymph node, ovaries (including oviducts), parathyroid glands, pancreas, pituitary gland, prostate, salivary glands, sciatic nerve, seminal vesicle, skeletal muscle, skin, spinal cord (cervical, thoracic, lumbar), spleen, sternum, stomach, testes, thymus, thyroid gland, tongue, trachea, ureter, urethra, urinary bladder, uterus, vagina and any other tissue showing changes.	
	Other		
104.4.12 Other examinations	Enzyme induction: At 3 months (all extra group animals) and at sacrifice (interim and final) 5 animals/sex/group were examined for enzyme induction in the liver, specifically: N-demethylase and		

		cytochrome P450. Fluoride content: At 3 months (all extra group animals) and at sacrifice (interim and final), the teeth and bones of 5 animals/sex/group were analysed for fluoride levels.
104.5	**Statistics**	Arithmetic group means and standard deviation were calculated for all quantitative results (except fluoride data). Test collective data were compared with control collective data using either Mann and Whitney or Wilcoxon's U test. Differences were considered significant at the 5% and 1% probability level. Data from the fluoride analysis were evaluated at a confidence level of 0.05. The Box test was used to test for homogeneity of variances between groups. If a difference was seen, a pairwise post-hoc comparison of the groups (one and two-tailed) was made using the Games and Howell modification of the Tukey-Kramer significance test. Comparison of survival curves used Wilcoxon's generalized test (Breslow test), a weighting proportional to respective group sized per event time.
104.6	**Further remarks**	
		105 RESULTS AND DISCUSSION
105.1	**Body weight**	No treatment related effects were seen in male animals. Female animals in the high dose group had a slight but significant increase in weight from week 1 to week 83..
105.2	**Food consumption**	No treatment related effects were seen.
105.3	**Water consumption**	No treatment related effects were seen.
105.4	**Clinical signs**	No treatment related effects were seen,
105.5	**Macroscopic investigations**	No treatment related effects were seen.
105.6	**Ophthalmoscopic examination**	Not applicable
105.7	**Haematology**	A number of miscellaneous statistically significant effects occurred which appear to have little toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal physiologic parameters for the effect. However, there is a suggestion of an effect on red cells—erythrocytes were reduced in high dose males, as were haemoglobin levels and haematocrit. Mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were reduced in some high dose males and females. Thrombocytes were increased in high dose males and females. There appeared to be no treatment related effect on white cells, with the possible exception of high dose group females at 24 months, which had an increased % of lymphocytes and decreased % of polymorphonuclear neutrophils (PMN).
105.8	**Clinical Chemistry**	A number of miscellaneous statistically significant effects occurred which appear to have little toxicological significance due to lack of dose response and/or lack of time dependence and which were within normal

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		physiologic parameters for the effect. However, cholesterol levels were significantly higher for high dose group males and females at all time points, for males and females in the 100 ppm dose group at interim sacrifice, and for females in the 100 ppm and 10 ppm dose groups at final sacrifice without clear dose-relationship.. Additionally, protein and albumin levels were significantly increased for females in all treatment groups at final sacrifice. Alkaline phosphatase was significantly increased in high dose groups at all time points.	
105.9	**Urinalysis**	Not applicable.	
105.10	**Pathology**	Moribund, 3-month and Interim autopsy: No treatment related effects were found. Final autopsy: Incidence of liver nodules was increased in females in the high dose group; no other treatment related effects were seen.	
105.11	**Organ Weights**	Absolute and relative liver weights were increased in males and females in the high dose groups. While other statistically significant changes were seen, they appear to have little toxicological significance as there is no apparent dose- or time-response.	
105.12	**Histopathology**	Interim autopsy: Hypertrophy of periacinal hepatocytes was seen in all males and more than half of the females in the high dose group. No other treatment related effects were seen. Final autopsy: Hypertrophy of periacinal hepatocytes was seen in more than half of the males and females in the high dose group. No other treatment related effects were seen.	X
105.13	**Other examinations**	Enzyme induction: No treatment related effects were seen. Fluoride incorporation: Fluoride levels in bones and teeth of male animals were statistically significantly increased in the 100 and 1000 ppm groups at both 12 and 24 months and in females animals in the 1000 ppm group.	
105.14	**Time to tumours**	Not applicable	
105.15	**Other**	Neoplastic lesions: No treatment related neoplastic lesions were seen at the interim autopsy. At the 24-month final autopsy a miscellany of benign and malignant tumours were seen an all groups (including controls) and were clearly not treatment related (lack of dose response, increased incidence in controls, single instance in middle dose group, etc). Female animals in the high dose group had a statistically significantly increased number of hepatocellular adenomas. Because of this, females in the high dose group also had a higher number of total and benign tumours.	

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106 APPLICANT'S SUMMARY AND CONCLUSION

106.1 Materials and methods

Groups of 60 male and female B6C3F1 mice were given NAK 4455 in the diet at concentrations of 0, 10, 100 and 1000 ppm for 12 months at which point 10 rats/sex/group were sacrificed (interim autopsy). The remaining 50 rats/sex/group were given NAK 4455 in the diet for an additional 12 months before sacrifice. Additionally, a further 10/animals/sex were treated for 13 weeks with either 0 or 1000 ppm NAK4455. Haematology, clinical chemistry, liver enzyme induction, measurement of fluoride levels, and gross and histopathology were performed on all animals at or just before sacrifice. Additionally, haematology and clinical chemistry and urinalysis were performed at 12, 18 (differential blood count only) and 24 months. This study fulfils the requirements of OECD 451 (1981) and US EPA FIFRA § 82-2 (1984), with the exception that an ophthalmoscopic examination was not performed. X

106.2 Results and discussion

Mortality was unaffected by treatment. No treatment induced changes in behaviour, or appearance were observed. No treatment related effects were seen on food or water consumption.

Body weights of females in the high dose group were statistically significantly increased (≤ 10%) over controls except during the last part of the study.

The results from the haematological and clinical chemistry studies combined with histopathology suggest that liver damage occur in both sexes exposed to 1000 ppm and may begin at 100 ppm in females.

In the high dose group, liver weights were increased and increased cholesterol levels were seen. In female animals increased incidence of liver nodes was seen. In male and female animals, increased hypertrophy of periacinal hepatocytes was seen at interim and final autopsy. High dose group females had increased levels of polymorphonuclear neutrophils, suggesting organ inflammation. High dose group females had increased levels of hepatocellular adenomas. This is not surprising given the liver damage that is apparently occurring at 1000 ppm and likely represent an epigenetic mechanism.

Also in the high dose group, there was an apparent decrease in haemoglobin and erythrocytes, particularly in male animals at interim sacrifice. Both males and females had increased thrombocytes in the high dose group.

A dose dependent increase in fluoride content in teeth and bones was seen starting at 100 ppm.

The lowest adverse effect level in this study is 100 ppm (equivalent to approximately 19.7 and 33.3 mg/kg bw-day for males and females respectively) based on liver damage in both sexes. The no observable adverse effect level is 10 ppm (equivalent to approximately 2.1 and 3.1 mg/kg bw-day for males and females respectively). This compound appears to cause benign liver adenomas in female animals at the 1000 ppm dose level (equivalent to 279 mg/kg-day). Based on the clear lack of a genotoxic mechanism, the propensity of mice to develop

		hepatoadenomas, and the lack of this response in another species, this compound does not present a carcinogenic risk to humans.	X
106.3	**Conclusion**		
106.3.1	Reliability	1	
106.3.2	Deficiencies	None	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 March, 2007
Materials and Methods	3.1.1.2 Purity of the compound is 94.4-95%. 3.4.11 For the 3-month extra group histopathology was only performed on liver. For the 12-month interim kill histopathology was only performed on kidneys, liver, thyroid and parathyroid, and altered organs and tissues. Otherwise the version of the applicant is acceptable.
Results and discussion	4.12 It is noted that in females of the high dose the increased incidences of haemangiosarcomas in the spleen (2/50), adenomas of the Harderian gland (8/50) and sarcomas of the subcutis (2/50) are above the historical control range. Otherwise the version of the applicant is adopted.
Conclusion	5.1 Urinalyses was not performed. 5.2 The RMS does not agree that it can be concluded from this study that transfluthrin does not present a carcinogenic risk to humans, nor that can be concluded that transfluthrin lacks carcinogenic responses in mice or other species. In the females at 1000 ppm (equal to 279 mg/kg bw/day) there may be a treatment-related increase in haemangiosarcomas in the spleen, adenomas in the Harderian gland and sarcomas of the subcutis. The incidences of these neoplastic lesions are above the historical control range and are considered possibly related to treatment. 28-03-2011 Based on new data the conclusion is adjusted, see discussion in Doc IIA. The incidences of hepatocellular adenomas and carcinomas in Table A6.5(02) -2 deviate from those reported in the original study see the right values under the table.
Reliability	1
Acceptability	acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.7(02) -1. Table for Haematology and Clinical Chemistry

Affected		0 ppm				10 ppm				100 ppm				1000 ppm			
Parameter, unit, sex		Months after start of treatment															
Haematology		3	12	18	24	3	12	18	24	3	12	18	24	3	12	18	24
Ery, 10^{12}/L	M	9.32	9.77		9.79		9.76		9.73		9.76		10.13	9.10**	9.37**		9.58
	F	8.67	9.42		8.46		9.69*		9.46		9.59		9.12	8.93	9.47		9.22
Hb, g/L	M	165	151		148		151		151		153		152	157**	149		146
	F	161	148		133		150		145		149		138	157	146		141
HCT, L/L	M	0.475	0.465		0.455		0.463		0.457		0.455		0.466	0.457**	0.438**		0.446
	F	0.472	0.422		0.406		0.457*		0.450		0.454		0.423	0.459*	0.446		0.435
MCH, pg	M	17.7	15.5		15.1		15.5		15.5		15.7		15.1	17.3*	15.9**		15.3
	F	18.6	15.7		15.9		15.5		15.4		15.6		15.2	17.6	15.5		15.3*
MCHC, g/L ery	M	348	325		325		327		330*		337**		326	344**	340**		329*
	F	341	335		327		329*		325		329**		327	341	328**		325
Thrombocytes, 10^9/L	M	911	891		1212		910		1271		907		1382**	980*	947		1425**
	F	763	779		658		852*		846**		823		741	880*	899**		871**
Lymphocytes, %	M	79.1	75.3	70.9	69.4		76.1	71.2	66.0		79.4	68.9	63.6	82.3	77.1	69.2	66.4
	F	83.1	76.6	69.2	69.5		82.1	71.1	72.2		83.0*	70.7	71.6	87.9*	81.8	74.5	86.8**
PMN, %	M	18.9	17.5	26.5	27.8		17.3	24.7	29.9		16.5	27.7	31.4	17.0	16.0	28.1	31.7
	F	15.5	17.0	26.3	27.3		12.3	24.2	22.5		12.8*	21.6	23.6	11.1	12.5*	20.6	11.1**
Clinical Chemistry																	
Months		3	12	24		3	12	24		3	12	24		3	12	24	
APh, U/L	M	128	87	105		130	86	103		126	92	119		137*	104**	139**	
	F	210	171	340		199	155	367		190	166	389		219	205**	741**	
Cholesterol, mM	M	2.87	3.22	3.08		2.85	2.98	3.02		2.97	3.58*	3.56		3.37**	3.69*	3.71*	
	F	2.36	2.16	2.17		2.52	2.38	3.41**		2.51	2.63**	3.72*		2.89**	2.85**	3.35**	
Protein, g/L	M	51.3	54.3	53.8		49.7	54.9	55.5		50.5	55.6	59.5		51.7	54.7	57.9	
	F	50.5	54.0	52.5		50.1	54.5	58.6		50.3	55.4	58.1*		51.0	55.2	58.3**	
Albumin, g/L	M	24.3	25.2	26.2		22.9	25.7	26.4		24.1	25.6	26.8		25.2	26.0	27.2	
	F	26.4	27.5	25.6		26.3	27.7	29.3**		26.6	28.4	28.8**		27.1	27.3	29.7**	
Fluoride, teeth, mg/g	M	0.259	0.494	0.584			0.538	0.651			0.798*	1.112**		1.301[a]	2.352**	2.349**	
	F	0.288	0.480	0.578			0.379	0.606			0.613	0.875		1.192[a]	2.032**	2.269**	
Fluoride, bone, mg/g	M	0.567	0.863	1.014			0.866	1.061			1.476**	1.824**		2.899[a]	4.428**	5.305**	
	F	0.506	0.790	0.962			0.783	1.098			1.338**	1.697		2.194[a]	3.843**	4.292**	

* p <0.05, ** p<0.01, [a] No statistical analyses performed for "extra groups" fluoride content, Ery = erythrocytes, Hb = haemoglobin, HCT = haematocrit, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, PMN = polymorphonuclear neutrophils, APh = alkaline phosphatase

Table A6.7(02) -2. Results from carcinogenicity study

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Number of animals examined	50	50	50	50	50	50	50	50		
Mortality	9	5	2	2	8	11	8	6	-	-
Clinical signs	-	-	-	-	-	-	-	-	-	-
Body weight	-	-	-	-	-	-	-	↑**	-	-
Food consumption	-	-	-	-	-	-	-	-	-	-
Overall tumour incidence (%):	50	58	42	54	50	56	40	74		
No. of animals with neoplasms	25/50	29/50	21/50	27/50	25/50	28/50	20/50	37/50	-	-
No. of animals with benign neoplasms	14/50	13/50	10/50	14/50	11/50	9/50	9/50	19/50	-	-
No. of animals with malignant neoplasms	10/50	10/50	9/50	11/50	11/50	16/50	7/50	10/50	-	-
No. of animals with > 1 neoplasm	3/50	9/50	3/50	5/50	6/50	6/50	7/50	17/50	-	-
Liver										
Hepatocellular adenoma	5/49	4/50	5/50	5/50	4/50	2/48	2/50	13/50*	-	-
Carcinoma	5/49	8/50	7/50	7/50	2/50	2/48	4/50	4/50	-	-
Non-neoplastic changes										
Nodule	10/50	7/50	13/50	4/50	13/50	5/50	12/50	15/50	-	-
Hypertrophy of periacinal hepatocytes (interim)	0/10	0/10	0/10	0/10	0/10	0/10	10/10	6/10	-	-
Hypertrophy of periacinal hepatocytes (final)	0/50	0/50	0/50	0/50	0/50	0/50	38/50	26/50	-	-
Absolute weight (interim 12 month)	-	-	-	-	-	-	↑**	↑**	-	-
Absolute weight (final 24 month)	-	-	-	-	↑*	↑	↑**	↑**	+	+

*p <0.05, ** p<0.01,- Not significantly different than control.

Right data

Parameter	Control data study		low dose		medium dose		high dose		dose-response + /	
	m	f	m	f	m	f	m	f	m	f
Liver										
Hepatocellular adenoma	5/49	4/50	4/50	2/48	5/50	2/50	5/50	13/50*	-	-
Carcinoma	5/49	2/50	8/50	2/48	7/50	4/50	7/50	4/50	-	-

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			107 REFERENCE
107.1	**1.1**	**Reference**	[redacted] (1988). Teratology study in the rat with NAK 4455. [redacted] [redacted] Report No. MTD0058. [BES Ref: MO-03-009816] Report date: February 12, 1988. Unpublished
107.2	**1.2**	**Data protection**	Yes
107.2.1	1.2.1	Data owner	Bayer CropScience
107.2.2	1.2.2	Companies with letters of access	
107.2.3	1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I.
			108 2 GUIDELINES AND QUALITY ASSURANCE
108.1	**2.1**	**Guideline study**	Yes, follows these guidelines established at the time: EPA New and Revised Health Effects Test Guidelines (1984), IRLG Recommended Guidelines (1981), and OECD Guidelines (1981). Also largely consistent with current guidelines: EC Method B.31 (Teratogenicity Test – Rodent and Non-Rodent), and OECD Guideline 414 (Prenatal Development Toxicity Study).
108.2	**2.2**	**GLP**	Yes
108.3	**2.3**	**Deviations**	Some specific information requested in current guidelines is not available, such as the time of day that clinical observations were performed. These specific deviations are listed in the appropriate region of the summary below. Although this study was done before current guidelines were established, the information provided is sufficient to give a valid conclusion in view of the lack of an effect of the test substance on the overall health outcome of both dams and foetuses. Also refer to section 3.5.
			109 3 MATERIALS AND METHODS

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3.1	**Test material**	NAK 4455 (transfluthrin)
109.1.1 3.1.1	Lot/Batch number	Batch No. 130187
3.1.2	Specification	As given in Section 2 of Doc IIIA.
3.1.2.1	Description	Brown liquid.
3.1.2.2	Purity	94.5%
3.1.2.3	Stability	Emulsions of the test article up to 5% concentration within aqueous Emulphor vehicle remained stable for at least 4 weeks.
3.2	**Test Animals**	
109.1.2 3.2.1	Species	Rat
3.2.2	Strain	Charles River Crl:CD BR strain
3.2.3	Source	Charles River Breeding Laboratories, Portage, Michigan, U.S.A.
3.2.4	Sex	Female and Male
3.2.5	Age/weight at study initiation	Approximately 13 weeks of age prior to breeding. bw males: 343-451 g; bw females: 211-290 g at time of insemination.
3.2.6	Number of animals per group	28 females/group; approximately half this number of males were available. Males and females were housed 1:2 during mating.
3.2.7	Control animals	Yes
3.2.8	Mating period	Until evidence of copulation was found (sperm observed in vaginal smear) for each individual female.
3.3	**Administration/ Exposure**	Oral, by gavage.
109.1.3 3.3.1	Duration of exposure	Days 6 – 15 post-insemination, for a total of 10 consecutive doses.
3.3.2	Post exposure period	4 days (rats sacrificed at presumed day 20 of pregnancy).
109.1.4		**Oral**
3.3.3	Type	Gavage
3.3.4	Dose levels	0 (Control), 25, 55 or 120 mg kg bw/day. Doses were chosen based on a range finding study. X 125
3.3.5	Vehicle	5% Emulphor EL-719 (polyethoxylated vegetable oil), 95% distilled water
3.3.6	Concentration in vehicle	0, 2.5, 5.5 or 12.5 mg/ml emulsion in 5% aqueous Emulphor.
3.3.7	Total volume applied	10 ml/kg
3.3.8	Controls	Vehicle, volume 10 ml/kg.
3.4	**Examinations**	

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109.1.5	3.4.1 Body weight	Yes, days 0, 6, 8, 10, 12, 15, and 20 during gestation.
	3.4.2 Food consumption	Yes, days 1, 6, 7, 12, 16, and 20 during gestation.
	3.4.3 Clinical signs	Dams were observed daily. General appearance and behaviour, body weight and food consumption.
	3.4.4 Examination of uterine content	Gravid uterine weight including the cervix. Number of corpora lutea counted. Number of implantations, including foetuses, resorptions, and implantation scars. The excised uterine horns were opened longitudinally and examined pressed between glass plates to assure that all tissue scars had been noted.
	3.4.5 Other maternal observations	Gross pathology at necropsy, including some organ weights (uterus, placenta, liver).
	3.4.6 Examination of foetuses	Each foetus was given a complete external examination and individual identification. The head was viewed from frontal, dorsal, and lateral aspects, pinnae and eye bulges noted for size and position. The palate was inspected for closure. The torso was examined for visceral herniation and irregular contours, limb position was noted, and the number of digits on all paws were counted. After external examination, one half of the foetuses from each dam were sacrificed by intracranial injection of barbiturate, and a complete internal stereoscopic examination was made of the abdominal and thoracic viscera. Following this examination these foetuses were fixed in Boüin's fixative and cut free-hand with razor blades for examination of the eyes and cranium. The remaining half of the foetuses were fixed intact in 70% ethanol. These foetuses were eviscerated and processed for ascertainment of skeletal abnormalities.
	3.4.6.1 General	Dam reproductive efficiency, fetal and placental weights, viability and sex ratios, and incidence of malformations/variations.
	3.4.6.2 Skeletal examination	Yes, approximately 50% of foetuses were processed according to the Alizarin Red-S method for clearing tissue and staining foetal bone, then evaluated for general skeletal development. Skull, vertebrae, ribs, pelvis, appendages, scapulae, clavicles, and sternum were examined and compared to the controls.
	3.4.6.3 Soft tissue	Yes, approximately 50% of foetuses were examined both externally and stereoscopically (viscera).
	3.5 Further remarks	EC B. 31 study guidelines suggest that food consumption be recorded at 3 day intervals and should coincide with days of body weight determination. This study did not follow this guideline, but food and body weight measurements were taken sufficient to determine that there was not a trend developing in treated animals different from controls. Also, the guideline suggests that clinical observations should be made and recorded at the peak period after dosing and at the same time each day. This study does not record that information; however, due to the

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		lack of overall effect of the test compound, the study is still valid. The guideline suggests that when examining foetuses, particular attention should be paid to the reproductive tract for signs of altered development. This study does not document an examination of foetal reproductive systems, but a 2 generation reproductive study (6.8.2 of this dossier) indicates that there are no reproductive consequences to exposure to transfluthrin.
3.6	**Statistics**	Statistical analysis of the data consisted of one or more of the following tests: Dunn (1964), Dunnett's (1955, 1964), Fischer's exact (Pagano-Halvorsen, 1981), Kruskal-Wallis (1952), and student's T test. Dam body weight and food consumption were compared to the control with Dunnett's test. Dam reproductive parameters were compared to control using Fishers' exact test (fertility and gestation index) and the Kruskal-Wallis and Dunn's tests (all other). Results were expressed using both the litter and the individual animal as the experimental unit.
	110 4	**RESULTS AND DISCUSSION**
110.1	**4.1 Maternal toxic Effects**	Overall health as measured by general appearance and behaviour, body weight, food consumption, and liver weight, was unaffected by transfluthrin administration, with the exception of tremors in 11% of mid-dose animals and 82% of high-dose dams that abated within a few hours, and the death of one high-dose dam. There was no treatment-related effect on body weight, food consumption, or gross pathology at any dose level. See Table 6.8.1-01 for summary numbers.
110.2	**4.2 Teratogenic / embryo-toxic effects**	Transfluthrin produced no statistically significant or toxicologically relevant adverse effect on any fetal parameter studied. Transfluthrin did not increase resorption nor promote late gestational death. There were no significant increases in the incidence of malformations or skeletal variations for any treatment group compared to the controls. See Table 6.8.1-01 for summary.
110.3	**4.3 Other effects**	None noted
110.4		
	111 5	**APPLICANT'S SUMMARY AND CONCLUSION**
111.1	**5.1 Materials and methods**	Previously non-treated, healthy, sexually mature male and female rats were used in this study following at least a 21-day acclimation period. 2 females were housed overnight with a breeder male, and vaginal smears confirmed copulation. The inseminated females were randomly assigned to 4 groups of 28 dams each. The females were given daily doses of 0, 25, 55, or 125 mg/kg transfluthrin in Emulphor vehicle, based on an earlier range finding study. Dosing volumes were based on body weight from Day 6 of gestation. The animals were dosed from days 6-15 of gestation, for a total of 10 consecutive doses. Dams were monitored for body weight

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			and food consumption throughout gestation. On day 20 the dams were sacrificed, and tissues examined. The ovaries were excised and corpora lutea counted. The uterus was removed and all fetuses, resorptions, and implants were noted. The viscera from the dams were scrutinized, the liver removed and weighed, and all gross pathological changes were recorded. Each foetus was removed from its amniotic sac, and viability determined. Each foetus was sexed and weighed, and its placenta weighed. A complete external examination was made of each foetus, including head, palate, torso, limbs and digits. Half the foetuses from each dam were used for a complete internal examination of the abdominal and thoracic viscera, and the other half were fixed and stained with Alizarin Red for examination of skeletal development. Treatment groups and controls were analyzed according to appropriate statistical methods. This study complies with the requirements of international guidelines.
111.2	**5.2**	**Results and discussion**	The only dose-related effect seen in the dams was transient tremor post-dosing; 11% mid-range dams and 82% of high-dose dams had tremors recorded during the test. One high dose dam also died on Day 8 (after 2 doses), and one time another dam was ataxic and salivated immediately following dosing. Otherwise, no overt clinical signs of transfluthrin toxicity were observed at any dose. Tremor is characteristic of pyrethroid exposure, and was an expected finding. Other measures, such as body weight, food consumption, necropsy findings, and reproductive efficiency, were normal. Parameters in the foetal treatment group were comparable to the controls. Foetal weights, viability, and sex ratios were not changed from controls. Placental weights were slightly but significantly greater than control for the high-dose group, but this increase is within the historical control range and was considered incidental. There were a few external and/or visceral changes observed in each treatment group as well as within the control group. The number of females pregnant at termination was fully adequate to meet current guideline requirements. No treatment-related changes were seen among pups, although the level of examination undertaken was in compliance with guideline requirements and was adequate to detect the usual range of variations. Transfluthrin was not teratogenic and showed no evidence of embryofoetal toxicity when tested up to levels that provoked tremors in the dams.
111.3	**5.3**	**Conclusion**	
111.3.1 maternal effects	5.3.1	LO(A)EL toxic	The LOAEL for maternal toxicity was 55 mg/kg bw/day, based on post-dosing tremor in 11% of pregnant females at this dose level.

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111.3.2	5.3.2	NO(A)EL maternal toxic effects	25 mg/kg bw/day.
111.3.3	5.3.3	LO(A)EL embryotoxic / teratogenic effects	Greater than 125 mg/kg bw/day, based on the absence of findings at the highest dose tested.
111.3.4	5.3.4	NO(A)EL embryotoxic / teratogenic effects	125 mg/kg bw/day.
111.3.5	5.3.5	Reliability	1
111.3.6	5.3.6	Deficiencies	Inconclusive evidence of toxicity at the top dose level. Doses and treatment-related effects in range finding study not discussed.

Evaluation by Competent Authorities	
111.4	**112 EVALUATION BY RAPPORTEUR MEMBER STATE**
112.1 Date	1-3-2007
112.2 Materials and Methods	In accordance with method OECD 414, groups of 23-25 pregnant rats were administered transfluthrin (purity 94.5%) daily at levels of 0, 25, 55 or 125 mg/kg bw orally by gavage during the 6th till 15th day of gestation. Animals were sacrificed at day 20.
112.3 Results and discussion	The version of the applicant is adopted. The critical endpoint for maternal toxicity was tremor occurring after dosing in mid-dose (11%) and high-dose (82%) dams, and death of one high-dose dam. No treatment-related effect on gestation or foetuses was detected.
112.4 Conclusion	NOAEL maternal toxicity = 25 mg/kg bw, based on post-dosing tremor in pregnant females. NOAEL embryotoxicity = 125 mg/kg bw, based on the absence of findings at the highest dose tested.
112.5 Reliability	1
112.6 Acceptability	Acceptable
112.7 Remarks	-
112.8	**113 COMMENTS FROM …**
113.1 Date	*Give date of comments submitted*
113.2 Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
113.3 Results and discussion	*Discuss if deviating from view of rapporteur member state*

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113.4	**Conclusion**	*Discuss if deviating from view of rapporteur member state*
113.5	**Reliability**	*Discuss if deviating from view of rapporteur member state*
113.6	**Acceptability**	*Discuss if deviating from view of rapporteur member state*
113.7	**Remarks**	

Table 6.8.1(01) -1 Teratogenicity Study in Rats – Summary of Results

Maternal findings					
Dose level, mg/kg bw/day		**0**	**25**	**55**	**125**
Number of females		28	28	28	28
Reproductive efficiency	Non-pregnant	3	5	3	3
	No. term litters	25	23	25	24
	Died during test	0	0	0	1
	Mean terminal bodyweight (corrected) (g)	383	383	373	379
	Mean gravid uterine weight (g)	87	88	83	84
	Median no. corpora lutea	16	15	16	16
	Median no. implantations	15	15	15	15
	Dams with >1 resorption	6	4	9	2
	Median litter size	14	14	15	14.5
	Median % male foetuses	56.3	46.7	46.7	53.1
	Median wt. viable foetuses (g)	3.8	3.8	3.8	3.7
	Median wt. of placentas (g)	0.52	0.55	0.54	0.56*
Median liver weight/100g body weight		4.85	4.73	4.68	5.01
Comment: * significantly different from control at 0.05 level					

Foetal findings									
Maternal dose level, mg/kg bw/day		**0**		**25**		**55**		**125**	
		L	F	L	F	L	F	L	F
No. of litters or viable foetuses		25	348	23	298	25	339	24	325
Litters/Foetuses with external malformations		0/25	0/348	0/23	0/298	0/25	0/339	0/24	0/325
Litters/Foetuses with visceral malformations		1/25	1/169	2/21	2/143	0/24	0/161	0/24	0/155
Litters/Foetuses with skeletal malformations		0/25	0/179	1/23	1/155	0/25	0/178	0/24	0/170
Litters/Foetuses with combined malformations		1/25	1/348	3/23	3/298	0/25	0/339	0/24	0/325
Litters/Foetuses with skeletal variations	Extra ribs	7/25	11/179	6/23	15/155	5/25	8/178	5/24	7/170
	Extra vertebrae/sacral shift	0/25	0/179	1/23	2/155	1/25	1/178	1/24	1/170
Comment: litters (L) and foetuses (F)									

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			114 REFERENCE
114.1	**1.1**	**Reference**	[redacted] (1989) NAK 4455 - Study for embryotoxic effects on rabbits after oral administration. [redacted]. [redacted] Report No. 18069 [BES Ref: MO-03-010420] Report date: June 8, 1989 Unpublished
114.2	**1.2**	**Data protection**	Yes
114.2.1	1.2.1	Data owner	Bayer CropScience
114.2.2	1.2.2	Companies with letters of access	
114.2.3	1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I
			115 2 GUIDELINES AND QUALITY ASSURANCE
115.1	**2.1**	**Guideline study**	Yes, follows these guidelines established at the time: EPA 83-3 "Teratogenicity Study" (1984), from Pesticide Assessment Guidelines Subdivision F, Hazard Evaluation: Human and Domestic Animals Also largely consistent with current guidelines: EC Method B.31 (Teratogenicity Test – Rodent and Non-Rodent), and OECD Guideline 414 (Prenatal Development Toxicity Study).
115.2	**2.2**	**GLP**	Yes
115.3	**2.3**	**Deviations**	Specific deviations are listed in the appropriate region of the summary below. Although this study is was done before current guidelines were established, the information provided is sufficient to give a valid conclusion at these dose levels in view of the lack of an effect of the test substance on the overall health outcome of both dams and foetuses. However, guidelines indicate that the highest dose level should produce observable maternal toxicity and the intermediate dose should produce minimal toxicity; the doses chosen in this experiment did not fulfil these requirements.

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Teratogenicity Study – Rabbit (translated from German original)

116 3 MATERIALS AND METHODS

3.6 Test material	NAK 4455 (transfluthrin)
116.1.1 3.1.1 Lot/Batch number	Mixed batch 250 987
3.6.2 Specification	As given in Section 2 of Doc IIIA.
3.6.2.1 Description	Brown-yellow clear liquid.
3.6.2.2 Purity	94.5% (27 Oct 1987), 95% (27 April 1988 retest)
3.6.2.3 Stability	Not specified other than that tests were made for stability and homogeneity in the administered formulation until 27 October 1988.
3.7 Test Animals	
116.1.2 3.2.1 Species	Rabbit
3.7.2 Strain	CHBB:Himalayan strain
3.7.3 Source	[redacted]
3.7.4 Sex	Female and Male
3.7.5 Age/weight at study initiation	Males were sexually mature and >2500 g. Females were sexually mature, nulliparous and 2231-3219 g.
3.7.6 Number of animals per group	15 females and males/group; current guidelines suggest 20 females.
3.7.7 Control animals	Yes, concurrent and historical controls.
3.7.8 Mating period	Mating was observed with one male and one female per cage. This was defined as day zero of gestation.
3.8 Administration/ Exposure	
116.1.3 3.3.1 Duration of exposure	Days 6 – 18 post-insemination, for a total of 13 consecutive doses.
3.8.2 Post exposure period	10 days (rabbits sacrificed at presumed day 29 of pregnancy).
116.1.4	**Oral**
3.8.3 Type	Gavage
3.8.4 Dose levels	0 (Control), 15, 50 or 150 mg kg bw/day. These doses are nearly identical to the doses used in the corresponding rat study. A pilot test using 200 mg/kg resulted in a 33% mortality rate.
3.8.5 Vehicle	5% Cremophor EL emulsion (glycerine-polyethylene glycol ricinoleate; non-ionic solubilizer made with ethylene oxide and castor oil), 95% distilled water
3.8.6 Concentration in vehicle	0, 0.3, 1.0 or 3.0% .

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3.8.7	Total volume applied	5 ml/kg bw	
3.8.8	Controls	Vehicle, volume 5 ml/kg bw	
3.9	**Examinations**		
116.1.5 3.4.1	Body weight	Day 0, then daily during dosing (days 6-18) and at day 29. Weight gain during gestation was documented.	
3.9.2	Food consumption	Monitored but data not given (reduced food intake noted in observations for some dams)	
3.9.3	Clinical signs	Dams were observed daily for appearance and behaviour.	
3.9.4	Examination of uterine content	At dam autopsy, determination of implantation count, corpora lutea count, uterine weight, and number of live and dead foetuses or embryos.	X
3.9.5	Other maternal observations	Gross pathology at necropsy. One high-dose and one midrange dose dam died during treatment.	
3.9.6	Examination of foetuses	At dam autopsy, foetuses were examined for determination of the sex of live foetuses, individual weight of live foetuses and runts, individual placenta weight, head/trunk length, external malformations, and skull malformations according to Wilson's method. Abdominal and thoracic organs were taken out and examined, then the foetus stained with alizarin red for appraisal of the bone system. Standardized methods established for examination of rat foetuses were followed for these rabbit foetuses.	
3.9.6.1	General	Dam reproductive efficiency.	
3.9.6.2	Skeletal examination	Yes, all foetuses were processed according to the Alizarin Red-S method for clearing tissue and staining foetal bone, then evaluated for general skeletal development. Malformations were reported.	
3.9.6.3	Soft tissue	Yes, all foetuses were examined externally and organs examined visually.	
3.10	**Further remarks**	EC B. 31 study guidelines suggest that food consumption be recorded at 3 day intervals and should coincide with days of body weight determination. This study did not follow this guideline, but food and body weight measurements were taken sufficient to determine that there was not a trend developing in treated animals different from controls. The guideline suggests that when examining foetuses, particular attention should be paid to the reproductive tract for signs of altered development. This study does not document an examination of foetal reproductive systems, but a 2 generation reproductive study (6.8.2 of this dossier) indicates that there are no reproductive consequences to exposure to transfluthrin.	X
3.7	**Statistics**	Wilcoxon's non-parametric rank sum test was used for weight gains, number of implantations, foetuses, and resorptions. Chi square tests were run for the number of runts and rates of fertilized and pregnant animals. Difference is considered significant if the probability of error is below 5%.	
		117 4 RESULTS AND DISCUSSION	
117.1	**4.1 Maternal**	Tremor occurred after dosing in one mid-dose and one high-dose dam, followed by death. The central nervous system symptoms seen in these	

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Teratogenicity Study – Rabbit (translated from German original)

toxic	**Effects**	two animals were consistent with pyrethroid exposure. Other effects were seen but they did not follow a dose-response curve that could indicate treatment-related effects. Overall health as measured by general appearance, behaviour, and body weight, which were unaffected by transfluthrin administration. Fertilization rate and gestation rate were not affected by transfluthrin.	
117.2	**4.2 Teratogenic / embryotoxic effects**	No treatment-related effect on the foetus was detected. The type and incidence of foetal malformations corresponded to the spectrum of malformations known to spontaneously occur in this strain, and have occurred previously within this laboratory.	
117.3 effects	**4.3 Other**	None noted	
117.4			
		118 5 APPLICANT'S SUMMARY AND CONCLUSION	
118.1 and	**5.1 Materials methods**	Previously non-treated, healthy, sexually mature male and female rabbits were used in this study following a 7-day acclimation period. Male rabbits were used only for breeding and were never treated. After an observed mating, the females were given daily doses of 0, 15, 50, or 150 mg/kg transfluthrin in Cremophor EL emulsion via stomach tube. The animals were dosed from days 6-18 of gestation, for a total of 13 consecutive doses. Dams were monitored for body weight and food consumption throughout gestation.	X
		On day 29 the dams were sacrificed, and tissues examined. The ovaries were excised and corpora lutea counted. The uterus was removed and all foetuses, resorptions, and implants were noted. Gross pathological changes were recorded. Viability of each foetus was determined, then it was sexed and weighed, and its placenta weighed. A complete external examination was made of each foetus, then the foetuses were inspected for visceral and skeletal malformations according to established protocols. Treatment groups and controls were analyzed according to appropriate statistical methods. This study complies with the requirements of current international guidelines.	
118.2 and	**5.2 Results discussion**	The only treatment-related effect seen in the dams was lethality in one mid-range dose animal and one high dose animal. Central nervous system symptoms seen prior to death were consistent with pyrethroid toxicity. Otherwise, no overt clinical signs of transfluthrin toxicity were observed at any dose. Other measures, such as body weight, fertility and gestation rates, and pathological alterations were within historical control ranges.	
		Parameters in the foetal treatment group were comparable to the controls. Foetal weights, viability, sex ratios, and malformations were not different from controls. There were a few external and/or visceral changes observed in each treatment group as well as within the control group, most commonly arthrogryposis.	X
		The number of females pregnant at termination was fewer than required	

			by current guidelines, but given the lack of significant effects, this number should be considered adequate. No treatment-related changes were seen among pups. The level of examination undertaken was in compliance with guideline requirements and was adequate to detect the usual range of variations. This laboratory has used this rabbit strain in previous studies and compared the current results with historical as well as concurrent controls. Transfluthrin was not teratogenic and showed no evidence of embryofoetal toxicity when tested up to levels that caused central nervous system effects and lethality in a small number of dams.
118.3	**5.3**	**Conclusion**	
118.3.1	5.3.1	LO(A)EL maternal toxic effects	The LOAEL for maternal toxicity was 50 mg/kg bw/day, based on one death with clinical symptoms consistent with pyrethroid toxicity of the central nervous system.
118.3.2	5.3.2	NO(A)EL maternal toxic effects	15 mg/kg bw/day.
118.3.3	5.3.3	LO(A)EL embryotoxic / teratogenic effects	Greater than 150 mg/kg bw/day, based on the absence of findings at the highest dose tested.
118.3.4	5.3.4	NO(A)EL embryotoxic / teratogenic effects	150 mg/kg bw/day.
118.3.5	5.3.5	Reliability	1
118.3.6	5.3.6	Deficiencies	Lack of consistent toxicity at the highest dose level (150 mg/kg) as requested by Guideline B. 31. However, the next higher dose tested (200 mg/kg) caused significant lethality in adult animals.

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Teratogenicity Study – Rabbit (translated from German original)

Evaluation by Competent Authorities	
118.4	**119 EVALUATION BY RAPPORTEUR MEMBER STATE**
119.1 Date	01-03-2007
119.2 Materials and Methods	In accordance with method OECD 414, groups of 12-15 pregnant rabbits were administered transfluthrin (purity 94.5%) daily at levels of 0, 15, 50 or 150 mg/kg bw, orally by gavage during the 6th till 18th day of gestation. Animals were sacrificed at day 29. **3.4.4; 3.5; 5.1:** Deviation from the OECD 414: In the original report, no data have been reported on food consumption other than whether or not the food intake was "low". The method states that dead and live foetuses are counted but no information on dead foetuses is given in the results. Stability of test substance in a 2 and 40 mg/mL solution in 0.5% aqueous Cremophor was meaured over only 4 days and decreased 1.9 and 5.2% respectively, in the course of this 4 days. Extrapolationg the 5.2% decrease in 4 days would yield a recovery of 82% after 13 days (number of days dosed).
119.3 Results and discussion	The version of the applicant is adopted The critical endpoint for maternal toxicity was tremor occurring after dosing in one mid-dose and one high-dose dam, followed by death. No treatment-related effect on gestation or foetuses was detected.
119.4 Conclusion	NOAEL maternal toxicity = 15 mg/kg bw, based on death of one dam in both the 50 and 150 mg/kg bw dosed groups, preceded by clinical symptoms consistent with pyrethroid toxicity of the central nervous. NOAEL embryotoxicity = 150 mg/kg bw, based on the absence of findings at the highest dose tested.
119.5 Reliability	2
119.6 Acceptability	Acceptable Due to lack of effects on all parameters measured other than death of 2 aminals, the lacking detailed data on food consumption do not compromise the validity of the study. As number of resorptions plus number of live foetuses is equal to the number of implantations, it is assumed that there were no dead foetuses.
119.7 Remarks	
119.8	**120 COMMENTS FROM …**
120.1 Date	*Give date of comments submitted*
120.2 Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
120.3 Results and discussion	*Discuss if deviating from view of rapporteur member state*
120.4 Conclusion	*Discuss if deviating from view of rapporteur member state*
120.5 Reliability	*Discuss if deviating from view of rapporteur member state*

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SECTION A6.8.1/02

BPD Data set IIA/ Annex Point VI.6.8.1

Teratogenicity Study – Rabbit (translated from German original)

120.6	**Acceptability**	*Discuss if deviating from view of rapporteur member state*
120.7	**Remarks**	

Table 6.8.1(02) -1 Teratogenicity Study in Rabbits – Summary of Results

Maternal findings					
Dose level, mg/kg bw/day		**0**	**15**	**50**	**150**
Number of females at start of test		15	15	15	15
Reproductive efficiency	Non-pregnant	3	2	1	0
	Mean weight gain during gestation (g)	156	216	174	193
Died during test		0	0	1	1
Reduced food intake during test		6	7	6	7
Little/soft stool		3	4	5	6
Cyst on Fallopian tube or uterus		0	4	2	1
Liver swelling or lobulation		0	1	1	1
Comment: Findings are listed when more than one animal was recorded with that finding. Listing does not imply a treatment-related effect.					
Foetal findings					
Maternal dose level, mg/kg bw/day		**0**	**15**	**50**	**150**
Individual malformations (number indicates number of animals with that malformation)		Arthrogryposis (1)		Cleft lip, lung hypoplasia, diaphragm hernia (1), Arthrogryposis (2)	Arthrogryposis, pigeon chest (1)
Comment: No alterations as a result of treatment were noted in corpora lutea count, number live foetuses, number of resorptions per litter, mean foetus or placental weight, head/trunk length, number of runts, foetuses with malformations or slight bone alterations, so those data are not presented.					

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Official use only

121 REFERENCE

121.1	**Reference**	[redacted] (1991) NAK 4455 technical (proposed c.n. Benfluthrin) - Multiple generation reproduction study in rats. [redacted] [redacted] Report No. T 9025165 [BES Ref: MO-03-010477] Report date: June 4, 1991. Unpublished
121.2	**Data protection**	Yes
121.2.1	Data owner	Bayer CropScience
121.2.2	Companies with letters of access	
121.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I

122 GUIDELINES AND QUALITY ASSURANCE

122.1	**Guideline study**	Yes, follows these guidelines established at the time: EPA Reproductive and Fertility Effects, Pesticide Assessment Guidelines 83-4 (1984), and OECD Guideline 416 Two generation reproductive toxicity study (1981). Also largely consistent with current guidelines: EC Method B.35 (Two-generation reproduction toxicity study, 2004), and OECD Guideline 416 (2001).
122.2	**GLP**	Yes
122.3	**Deviations**	Lack of explanation of statistical methods, lack of emphasis on reproductive tract (such as no sperm analysis or saved samples, oestrus cycle not monitored), and growth and development of the reproductive systems. There is no indication that mating or functional behaviors were monitored. However, the study contained more than 20 litters in each generation, there were two F1 and F2 generations, and the animals were sufficiently monitored to determine that there was no decrease in reproductive potential after administration of transfluthrin across full reproductive cycles in non-prenatally exposed animals, as well as in animals exposed throughout development.

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123 MATERIALS AND METHODS

123.1	**Test material**	As given in section 2, NAK 4455 (current name transfluthrin, suggested name benfluthrin in report)
123.1.1	Lot/Batch number	Batch no. 250987
123.1.2	Specification	As given in Section 2 of Doc IIIA.
123.1.2.1	Description	brown solid.
123.1.2.2	Purity	94.4 – 95.3%
123.1.2.3	Stability	Analyzed 4 times, listed as stable for 3 weeks in the diet.
123.2	**Test Animals**	
123.2.1	Species	Rat
123.2.2	Strain	Wistar/HAN (Kfm: WIST, outbred, SPF quality).
123.2.3	Source	[redacted]
123.2.4	Sex	Male & Female
123.2.5	Age/weight at study initiation	P group animals were aged approximately 4 weeks at delivery to the test site, and were approximately 5-6 weeks at the start of test compound administration. The animals were approximately 17 weeks of age at pairing. The weight range at beginning of administration was 124-173 g for males, 90-132 g for females.
123.2.6	Number of animals per group	Each group in the P generation consisted of 30 male and 30 female rats, and each group of the F1 parent generation consisted of 26 male and 26 female rats.
123.2.7	Duration of mating	Males and females were kept together for 21 days or until vaginal smears showed evidence of mating. If no evidence was found by 21 days, a second male with a history of successful mating was paired with the female for a maximum of 5 days.
123.2.8	Deviations from standard protocol	Both the P and F1 generations littered twice; the first litter was used for tissue preservation and examination of macropathology. The second litter was used as parents of the next generation, and for macropathology. Litter size was standardized at 8 pups on day 4 post partum.
123.2.9	Control animals	Yes
123.3	**Administration/ Exposure**	
123.3.1	Animal assignment to dosage groups	Equal numbers of P animals were randomly assigned to each dose group. F1 animals continued in the same dose group as the dam.

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123.3.2	Duration of exposure before mating	The parental generation was dosed for 84 days before pairing, and throughout pairing, gestation, and lactation of the F1 litters (F1A and F1B). Following weaning of the F1B litters on postnatal day 21, animals selected for the next parental generation were dosed beginning at 5-6 weeks of age. The F1 animals were treated for 105 days prior to pairing and throughout pairing, gestation, and lactation of the F2A and F2B litters as before.
123.3.3	Duration of exposure in general P, F1, F2 males, females	P animals were dosed after 10 days of acclimatization at the testing facility, then through prepairing, pairing, gestation and lactation of the females for two litters, and until sacrifice after the weaning of the second litter. F1 animals were exposed to the a.s. throughout their lifespan, including prepairing, gestation, lactation, and as adults if selected to be the parental generation (sacrificed after weaning of the second litter). F2 animals were exposed to the a.s. until sacrifice at weaning.
		Oral
123.3.4	Type	In food, mixed into a microgranulated diet and pelleted.
123.3.5	Concentration	0 (Control), 20, 200 or 1000 ppm in diet; Food consumption was ad libitum. Range of test article intake, see Table 6.8.2-01.
123.3.6	Vehicle	The test article was dissolved in acetone and mixed with granulated food. Water was added to the granulated food for the test and control diets to allow pelleting of the food.
123.3.7	Concentration in vehicle	a.s. concentration in acetone not relevant for final concentration in food: 20, 200, 1000 ppm
123.3.8	Total volume applied	Not applicable
123.3.9	Controls	Plain diet, granulated diet processed into pellets similarly to test article diet.
123.4	**Examinations**	
123.4.1	Clinical signs	Animals were monitored daily for morbidity and mortality. Clinical biochemistry data from blood and liver were taken from P and F1 parental animals at sacrifice.
123.4.2	Body weight	The body weight and weight gain of all animals was monitored. Mean body weight of groups was recorded.
123.4.3	Food/water consumption	Food consumption was monitored in all animals, but water intake was not.
123.4.4	Oestrus cycle	No
123.4.5	Sperm parameters	No

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123.4.6	Offspring	See Table 6.8.2-01 and -02 for numbers Looked for number and sex of pups - Yes stillbirths - Yes live births - Yes presence of gross anomalies - Yes weight gain - Yes physical or behavioural abnormalities - No
123.4.7	Organ weights P and F1	Organ weights were taken for kidneys, liver, spleen, testes, and ovaries. Males that failed to induce pregnancy had epididymides, prostate and seminal vesicles removed and weighed.
123.4.8	Histopathology P and F1	P parent animals – shortly after F1B pups were weaned, all high dose and control P adults were sacrificed and examined macroscopically. Samples of the following tissues were collected and fixed: all gross lesions, kidneys, liver, ovaries, pituitary gland, prostate, seminal vesicles with coagulating gland, spleen, testes with epididymides (males), uterus and cervix, and vagina (females) were taken from all adults, weighed, and preserved for histopathology. The uteri of apparently non-pregnant females after both F1A and F1B matings were placed into a solution of ammonium sulphide to visualize possible hemorrhagic alterations of implantation sites. The testes with epididymides, prostate and seminal vesicles from all males failing to induce pregnancy during the second pairing were weighed and examined histopathologically. F1 parent animals – shortly after F2B weaning, all high dose and control F1 adults were sacrificed and examined macroscopically. Specified organs were weighed and tissue preserved for histopathology. The uteri of apparently non-pregnant females after both F2A and F2B matings were placed into a solution of ammonium sulphide to visualize possible hemorrhagic alterations of implantation sites. The testes with epididymides, prostate and seminal vesicles from all males failing to induce pregnancy during the second pairing were weighed and examined histopathologically. The thyroid glands from F1 parents and F2B litters were also examined.
123.4.9	Histopathology F1 not selected for mating, F2	One male and one female per litter were selected for histopathology on day 21 post partum as described for the P and F1 adults. All other pups were sacrificed close to day 21, examined macroscopically, and discarded.
123.5	**Further remarks**	Brain and thymus were not weighed a suggested in current guidance. No treatment-related effects were noted in any tissue weighed.

124 RESULTS AND DISCUSSION

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124.1	**Effects**		
124.1.1	Parent males	At 1000 ppm, increased kidney weights were noted, corresponding to microscopic findings, and liver weights were also increased; these were considered treatment-related. Microscopic examination showed increased incidence of basophilic tubules and tubular casts in the kidneys.	X
124.1.2	Parent females	At 1000 ppm, increased kidney weights were noted, corresponding to microscopic findings; this was considered treatment-related. Microscopic examination showed increased incidence of tubular pigments and pelvic calcinosis in the kidneys.	X
124.1.3	F1 males	No treatment-related abnormalities were observed at external examination at birth. The sex ratio in all dose groups was not affected by treatment when compared to the control group. A slight decrease in liver triglycerides was seen at 1000 ppm, and was considered to be treatment-related. Microscopic examination showed increased incidence of basophilic tubules and tubular casts in the kidneys at 1000 ppm..	X
124.1.4	F1 females	No treatment-related abnormalities were observed at external examination at birth. The sex ratio in all dose groups was not affected by treatment when compared to the control group. Mean body weights were reduced in the 20 ppm group throughout the lactation period, but the effect was not dose-related. Slight retardation of body weight gain was seen in the high dose (1000 ppm) animals during the first part of the prepairing period, and was considered to be treatment-related. A slight decrease in liver triglycerides was seen at 1000 ppm, and was considered to be treatment-related. Microscopic examination showed increased incidence of tubular pigments and pelvic calcinosis in the kidneys at 1000 ppm. Slight decrease in body weight gain was observed.	
124.1.5	F2 males	No treatment-related abnormalities were observed at external examination at birth. The sex ratio in all dose groups was not affected by treatment when compared to the control group. The body weights were all within the normal range and not affected by treatment with the test article.	
124.1.6	F2 females	No treatment-related abnormalities were observed at external examination at birth. The sex ratio in all dose groups was not affected by treatment when compared to the control group. The body weights were all within the normal range and not affected by treatment with the test article.	

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124.2	**Other**	The following parameters were not considered to be affected by treatment:	
		Viability, general behaviour and appearance of the P and F1 parent animals.	X
		Food consumption and body weight gain, except for females of F1 generation, 1000 ppm, during prepairing period.	
		Reproduction parameters in both P and F1 generations (fertility index, conception rate, gestation index, mean precoital time, gestation duration, mean number of living or dead pups at first litter check, postnatal loss, and breeding loss).	
		Teratogenesis	
		Sex ratios, organ weights, mean body weights, and weight gain in all groups of pups (F1A and B, F2A and B).	
		Clinical biochemistry, except for slight increase in liver triglycerides of F1 parent animals at 1000 ppm.	X
		Organ weights of parent animals, except for the P generation at 1000 ppm. Macroscopic examination of all animals, and microscopic examination of F2B pups.	

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125 APPLICANT'S SUMMARY AND CONCLUSION

125.1 Materials and methods

Animals were housed individually under standard laboratory conditions, with males and females in cages next to each other to promote normal oestrous cycling in the females. The test article was administered in the diet in three doses, 20, 200, and 1000 ppm, and food consumption and weight gain was tracked in all animals throughout the study. Animals were checked daily and data related to animal health was recorded.

Thirty Parental generation animals were received as young adults, and fed the test article for 84 days to ensure exposure to the test article for a full oestrous/sperm production cycle before mating. P animals were randomly paired, and monitored for evidence of mating. Once evidence of mating was found, the females were housed individually again for the period of gestation. The first litters of pups born, the F1A generation, were externally examined at birth, monitored for 21 days during the lactation period, and data recorded for each animal and the litter as a whole (numbers born, live/dead, gender, weight). At 21 days one male and one female pup were processed for histological examination, and the other pups sacrificed. The P females were rested for 10 days and again paired with a male of that generation to produce a second litter of pups as before, the F1B generation. The F1B animals were raised to weaning at 21 days, then one male and one female from each litter (26 of each) were selected to be the F1 parent generation. All P adults were sacrificed shortly after day 21 postpartum of the F1B generation, and examined macroscopically. Specified organs were taken for weighing and histopathological examination. Additionally, blood and liver samples were taken from 10 animals per sex and treatment group for clinical biochemistry. Uteri and testes from unsuccessful breeding animals were examined histopathologically.

The twenty six pairs of F1 animals were fed the test diet for 105 days prior to pairing, then treated as above to derive the F2A and F2B generations, and a record of pup growth until weaning. The F2A generation was again sacrificed at 21 days and one male and one female of each litter examined histopathologically. The F2B animals were raised to weaning, and a male and a female from each litter preserved for histopathology. All other F2 pups were sacrificed and discarded. The F1 parental animals were sacrificed shortly after day 21 postpartum for the F2B pups, and examined for macroscopic and histopathological effects, and for clinical biochemistry as in the P generation.

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125.2 Results and discussion	No signs of reaction to treatment were noted during the experimental period in adults or pups. Indices of animals with macroscopically abnormal conditions did not indicate a test article-related effect. Similar food consumption and weight gain in all groups indicated general good health.	
	Tissue analysis of adult animals in the 1000 ppm treatment group showed effects on the kidneys and liver, consistent with findings described elsewhere in Section 6 (Mammalian toxicology). These effects did not decrease the reproductive potential of the animals.	X
	The LOAEL is determined to be 1000 ppm based on treatment-related effects on the kidney and liver of adult animals; the NOAEL from this study is therefore 200 ppm. The lack of effect on reproductive parameters at 1000 ppm may indicate that the test article is non-toxic to developing animals at a higher dose than would be allowable for chronic exposure in adult animals.	X
125.3 Conclusion	No teratogenic effect was observed by external examination of the pups in any group of either generation. There was no deleterious toxicological effect of the test article on the growth and reproductive performance of multiple generations in the Wistar/Han rat.	
125.3.1 LO(A)EL		
125.3.1.1 Parent males	1000 ppm (range of 45-191 mg/kg bw/day) - increased kidney weights and cellular abnormalities, increased liver weights in P generation males	X
125.3.1.2 Parent females	1000 ppm - increased kidney weights and cellular abnormalities	X
125.3.1.3 F1 males	1000 ppm - slight decrease in liver triglycerides.	X
125.3.1.4 F1 females	1000 ppm - based on slightly decreased body weight gain in F1 females, and slight decrease in liver triglycerides.	X
125.3.1.5 F2 males	No effects noted at any dose.	
125.3.1.6 F2 females	No effects noted at any dose.	
125.3.2 NO(A)EL		
125.3.2.1 Parent males	200 ppm (range 9-38 mg/kg bw/day) for general tolerability, and 1000 ppm (range 45-191 mg/kg bw/day) for reproduction.	X
125.3.2.2 Parent females	200 ppm (range 9-38 mg/kg bw/day) for general tolerability, and 1000 ppm (range 45-191 mg/kg bw/day) for reproduction.	X
125.3.2.3 F1 males	200 ppm (range 9-38 mg/kg bw/day) for general tolerability, and 1000 ppm (range 45-191 mg/kg bw/day) for reproduction.	X
125.3.2.4 F1 females	200 ppm (range 9-38 mg/kg bw/day) for general tolerability, and 1000 ppm (range 45-191 mg/kg bw/day) for reproduction.	X
125.3.2.5 F2 males	1000 ppm (range 45-191 mg/kg bw/day) for growth.	
125.3.2.6 F2 females	1000 ppm (range 45-191 mg/kg bw/day) for growth.	
125.3.3 Reliability	2	

Doc. IIIA Section A6.8.2 BPD Data set IIA/ Annex Point IIA6.8.2	Multigeneration Reproduction Oral Toxicity Study in Rats
125.3.4 Deficiencies	This study does not discuss of the results of the range-finding study used to determine the doses used in this study. The current guidance emphasizes examination of the reproductive organs, and reproductive behaviours, which are not included in this study. Nonetheless, the purpose of the study is to determine whether the reproductive performance of the rat is affected by the test article, and the experimental design and execution are strong enough to determine that overall reproductive performance is not altered by exposure to the test article at up to 1000 ppm in the diet.

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Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	3-4-2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	4.1.1. In addition, microscopic examination showed increased incidence and severity in tubular pigment in the kidneys. Microscopic changes were seen in all treated dose groups. In the prostate, inflammatory cells are observed more frequently in the high dose than in the control group. Prostates should have been examined in all animals from intermediate dose groups, too.
	4.1.2 Also an increased incidence in tubular casts and basophilic tubules in the kidneys is observed in all treated dose groups. The increase in incidence of pelvic calcinosis is not dose dependent.
	4.1.3 In addition, an increased incidence in tubular pigment is seen in the kidneys at 1000 ppm. Kidneys should have been examined in all animals from intermediate dose groups, too. In the prostate, inflammatory cells are observed more frequently in the high dose than in the control group. Prostates should have been examined in all animals from intermediate dose groups, too.
	4.1.6 A slight increased incidence in basophilic tubules in the kidneys is observed at 1000ppm. Kidneys should have been examined in all animals from intermediate dose groups, too.
	4.2 At 1000 ppm, number of total litter loss was increased; 2 dams of the P generation had total litter loss (F1A).
	The mentioned "increase" in liver triglycerides of F1 actually is a "decrease" in liver triglycerides.
	5.2 Effects on the kidneys were present in all treated groups, including the lowest dose group (20 ppm, range 1-2 mg/kg bw/day) of the P-generation. Therefore, it is not possible to establish a NOAEL for parental toxicity.
	The LOAEL is determined to be 20 ppm based on treatment-related effects on the kidney of adult animals.

Conclusion	5.3.1 LO(A)EL: Parent males: 20 ppm (range 1-2 mg/kg bw/d), based on increased incidence in tubular pigment and basophilic tubules in kidneys. Parent females: 20 ppm (range 1-2 mg/kg bw/d), based on increased incidence in tubular pigment and tubular casts in kidneys. F1 males; general toxicity: can not to be established as kidneys of the low and mid-dosed groups have not been examined; developmental toxicity: 1000 ppm (45-191 mg/kg bw/day), based on increased incidence of total litter loss F1 females; general toxicity: can not to be established as kidneys of the low and mid-dosed groups have not been examined; developmental toxicity: 1000 ppm (45-191 mg/kg bw/day), based on increased incidence of total litter loss Conclusion on F2 is agreed on; No effects noted at any dose. 5.3.2 NO(A)EL parent males: general tolerability: <20 ppm (<1-2 mg/kg bw/day), 1000 ppm (45-191 mg/kg bw/day) for reproduction. parent females: general tolerability: <20 ppm (<1-2 mg/kg bw/day), 1000 ppm (45-191 mg/kg bw/day) for reproduction. F1 males and females : general toxicity: can not to be established as kidneys of the low and mid-dosed groups have not been examined; developmental toxicity: 200 ppm (9-38 mg/kg bw/d). F2 males and females: 1000 ppm (range 45-191 mg/kg bw/day) for growth. 22-3-2011: TM I2011 considers the histopathological effects in the kidney as no true adverse effects. To study effects on the kidney a repeated dose study will be more appropriate (see appendix written by the applicant, see also 6.5.01).
Reliability	2
Acceptability	Acceptable. Kidneys should also have been examined in all F1 animals from other dose groups, as this organ showed abnormalities. However, the kidney is not a reproduction organ and in the P generation, even the lowest dose was even an effect dose. No behavioral and physical observations of F1 and F2 pups have been reported.
Remarks	Pups from the 2 litters lost died perinatally (i.e. pups were born dead or died in the first 3 days post parturition) could result in labeling of transfluthrin with R63 (Possible risk of harm to the unborn child) , however the transflutrhin DNT study confirms that at dose levels in the range and above the highest dose level tested in the multiple generation reproduction study the viability of the fetuses is not affected by transfluthrin. Therefore, labeling is not proposed
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*

Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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Table A6.8.2-01 Reproductive toxicity study, Parental generations .

Parameter		Generation	control m	control f	20 ppm m	20 ppm f	200 ppm m	200 ppm f	1000 ppm m	1000 ppm f
Mortality	incidence	P	0/30	1/30	0/30	0/30	0/30	0/30	1/30	1/30
		F_1	0/26	0/26	0/26	0/26	1/26	0/26	0/26	0/26
Comment: - indicates no effect in the following table										
Test Article Intake Range, mg/kg/day	Males – general Females – prepairing and gestation	P	0	0	1-2	1-3	9-24	13-25	48-126	66-125
		F_1	0	0	1-3	1-3	9-26	12-25	45-131	62-124
	Females - lactation	P	-	0	-	2-4	-	21-38	-	119-191
		F_1	-	0	-	2-4	-	21-37	-	103-188
Clinical Biochemistry	Cholinesterase activity, P450 liver content, liver N-demethylase, and 19 other blood and liver measurements.	P	-	-	-	-	-	-	-	-
		F_1 (change in liver triglycerides only)	-	-	-	-	-	-	18% decrease, not sig.	32% decrease, *p<0.01
Organ weights (summary numbers not compiled Inc=increase))	Kidney	P	-	-	-	-	-	-	Inc.	Inc
	Liver		-	-	-	-	-	-	Inc.	-
	Spleen		-	-	-	-	-	-	-	-
	Testes/Ovaries		-	-	-	-	-	-	-	-
	Kidney	F_1	-	-	-	-	-	-	-	-
	Liver		-	-	-	-	-	-	-	-
	Spleen		-	-	-	-	-	-	-	-
	Testes/Ovaries		-	-	-	-	-	-	-	-

Continued

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Multigeneration Reproduction Oral Toxicity Study in Rats

Reproductive Performance										
Mean precoital time		P, F1A	3.9		2.9		2.5		2.5	
		F1B	2.9		3.1		2.8		2.6	
		F_1, F2A	3.5		2.8		4.0		3.6	
		F2B	2.3		2.8		4.6		3.5	
Percentage mating		P, F1A	100	100	100	100	100	100	100	100
		F1B	100	100	100	100	100	100	100	100
		F_1, F2A	100	100	100	100	100	100	100	100
		F2B	100	100	100	100	100	100	100	100
Fertility index (%)	(same as conception rate)	P, F1A	100		90		100		96.6	
		F1B	100		100		100		86.2	
		F_1, F2A	80.8		92.3		92.3		92.3	
		F2B	76.9		100		88.5		84.6	
Gestation index (%)		P, F1A		100		100		100		100
		F1B		100		100		100		100
		F_1, F2A		100		100		100		100
		F2B		95.0		96.2		95.7		100
	Number of females mated/pregnant/ rearing pups as used for litter data	**P generation**, F1A	30/30/30		30/27/27		30/30/30		29/28/26	
		F1B	29/29/29		30/30/30		30/30/30		29/25/25	
		F_1 generation, F2A	26/21/21		26/24/24		26/24/24		26/24/24	
		F2B	26/20/19		26/26/25		26/23/22		26/22/22	

Continued

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Multigeneration Reproduction Oral Toxicity Study in Rats

Litter size, pup survival of dams rearing pups (litters were culled to 8 pups on day 4)	Mean, living at first check	**P**, F1A	11.6	10.9	10.9	11.5
		F1B	11.6	10.0	10.9	11.8
		F_1, F2A	10.0	9.5	11.0	12.3
		F2B	9.6	10.3	10.7	10.7
	Mean, dead at first check	**P**, F1A	0.13	0.15	0.13	0.08
		F1B	0.07	0.13	0.03	0.0
		F_1, F2A	0.05	0.21	0.29	0.21
		F2B	0.05	0.28	0.23	0.23
	% postnatal loss days 0-4	**P**, F1A	0.9	1.7	1.8	1.0
		F1B	0.3	2.3	0.6	0.3
		F_1, F2A	1.9	3.0	1.5	1.0
		F2B	0.0	1.2	0.4	2.5
	% postnatal loss days 4-21	**P**, F1A	1.3	0.5	1.8	1.5
		F1B	0.4	1.8	0.9	0.5
		F_1, F2A	2.6	2.3	0.6	1.0
		F2B	0.0	1.6	0.6	0.6
Litter weight	Mean	Summary data given in graph form in the report; there was no treatment-related effect, and male and female pups were similar.				
Pup weight	Mean	Summary data given in graph form in the report; there was no treatment-related effect, and male and female pups were similar.				
Survival index	*(% found dead at first check)*	P, F1A	0.13	0.15	0.13	0.08
		F1B	0.07	0.13	0.03	0.0
		F_1, F2A	0.05	0.21	0.29	0.21
		F2B	0.05	0.28	0.23	0.23

Continued

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Multigeneration Reproduction Oral Toxicity Study in Rats

Table A6.8.2-02. Reproductive toxicity study, Pups before weaning for dams rearing the pups

Parameter		Generation	control m	control f	20 ppm m	20 ppm f	200 ppm m	200 ppm f	1000 ppm m	1000 ppm f
Mortality	Overall	F_1 A	3	3	3	3	3	7	2	4
		F_1 B	-	2	5	6	3	1	1	1
		F_2 A	3	5	6	5	3	2	3	2
		F_2 B	-	-	3	3	1	1	4	3
	First day	F_1 A	-	-	-	-	1	2	1	-
		F_1 B	-	1	4	4	-	-	-	-
		F_2 A	-	1	3	1	-	-	-	-
		F_2 B	-	-	-	1	-	-	-	1
Sex Ratios, % male	(After parturition)	F_1 A	50.1		50.0		50.8		47.0	
		F_1 B	50.4		56.8		48.3		55.6	
		F_2 A	48.3		50.6		52.1		49.8	
		F_2 B	54.4		48.6		53.4		50.8	
Organ Weights		There were no test article related changes in the organ weights of any of the pups.								

Appendix

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Transfluthrin Tech. – BES's position on the derivation of the Acceptable Exposure Level for systemic chronic exposure

In this study, transfluthrin was administered in the diet at the dose levels of 0, 20, 200, and 1000 ppm. Thirty parental generation animals (P) were treated for 84 days to ensure exposure to the test article for a full oestrous/sperm production cycle before mating and were then randomly paired and monitored for evidence of mating. The mated females were housed individually during their period of gestation and their litters constituted the F1A litters which were monitored for 21 days during the lactation period. On post-natal day (PND) 21 all of the F1A animals were sacrificed. The P females were rested for 10 days and again paired with a male of that generation to produce a second litter, the F1B generation. The F1B litters were raised to weaning at PND 21, and one male and one female from each litter (26 of each sex/group in total) were selected to be the F1 parent generation. All P adults were sacrificed shortly after day 21 postpartum of the F1B litters. The twenty six animals/sex/group of the F1 generation were fed with treated diet for 105 days prior to pairing, then treated as above to derive the F2A and F2B litters. The F2A litters were sacrificed on PND 21. The F2B animals were raised to weaning. The F1 parental animals were sacrificed shortly after day 21 postpartum for the F2B pups.

With respect to the kidneys, histopathology was performed at the high dose and for control animals of the P and F1 animals selected for pairing, for animals killed *in extremis* or which died during the study, and for one male and one female pup of each F2B litter. Additionally, histopathology of the kidney was also performed at the low-and mid dose in the P animals. Results are presented in the following tables 1 to 3:

Table 1: Histopathology of the kidneys in the P animals [redacted], 1991).

Sex	Males				Females			
Dose level (ppm)	**0**	**20**	**200**	**1000**	**0**	**20**	**200**	**1000**
Examined kidneys	30	30	30	29	29	30	30	30
Tubular pigment								
Grade 1	0	1	4	5	2	9	11	13
2	0	0	0	9	0	0	1	10
3	0	0	0	0	0	0	1	0
Total	0	1	4	14	2	9	13	23
Basophilic tubules								
Grade 1	3	8	12	10	0	4	2	3
2	1	3	2	3	0	0	0	1
Total	4	11	14	13	0	4	2	4

Grade 1 = minimal, 2 = slight, 3 = moderate

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Transfluthrin Tech. – BES's position on the derivation of the Acceptable Exposure Level for systemic chronic exposure

Table 2: Histopathology of the kidneys in the F1 animals ([redacted], 1991).

Sex	Males				Females			
Dose level (ppm)	**0**	**20**	**200**	**1000**	**0**	**20**	**200**	**1000**
Examined kidneys	26	6	6	26	26	6	0	26
Tubular pigment								
Grade 1	0	0	0	4	8	1	0	16
2	0	0	0	1	0	0	0	4
Total	0	0	0	5	8	1	0	20
Basophilic tubules								
Grade 1	7	3	2	11	6	0	0	4
2	6	0	1	7	1	0	0	0
3	0	0	1	1	0	0	0	0
Total	13	3	4	19	7	0	0	4
Pelvic calcinosis								
Grade 1	5	2	1	2	6	1	0	11
2	1	1	1	2	1	0	0	3
3	1	0	0	0	1	0	0	0
Total	7	3	2	4	8	1	0	14
Tubular casts								
Grade 1	6	2	2	14	3	1	0	3
2	8	0	1	6	1	1	0	0
3	0	0	1	1	1	0	0	0
Total	14	2	4	21	5	2	0	3

Grade 1 = minimal, 2 = slight, 3 = moderate

Table 3: Histopathology of the kidneys in the F2B animals ([redacted], 1991).

Sex	Males				Females			
Dose level (ppm)	**0**	**20**	**200**	**1000**	**0**	**20**	**200**	**1000**
Examined kidneys	19	4	1	21	18	3	2	21
Basophilic tubules								
Grade 1	11	1	0	4	5	2	1	9
Total	11	1	0	4	5	2	1	9

Grade 1 = minimal

In the F generation the increase in renal tubular pigment was more pronounced in the females where only two control rats were affected compared to twenty three in the high dose group. In males, no control rats had this finding, compared to fourteen in the high dose group.

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Transfluthrin Tech. – BES's position on the derivation of the Acceptable Exposure Level for systemic chronic exposure

The severity grade increased from minimal to slight in high dose males and in mid and high dose females. One mid dose female had a moderate degree of severity. In the FI generation this finding occurred in twenty high dose females compared to eight controls and in five high dose males compared to zero in controls. The low-and mid doses were not fully assessed. This pigment, which from its microscopic appearance can be assumed to be lipofuscin, often referred to as "wear and tear" pigment, is a normal component of the cortical tubules of older rats. It is derived from the oxidation of unsaturated tissue lipids or lipoproteins. It is therefore considered not to be an adverse effect but a consequence of the aging process.

Basophilic cortical tubules, represent tubules with altered epithelial cells, which can be a sequel to degenerative conditions or due to increased cell turnover. As such they may reflect stages of regeneration or atrophy. In this study, treatment with the test article resulted in an increased incidence of basophilic tubules in males of both P and F1 generations, whereas treated females of these generations only recorded a low incidence of this finding. However, the degree of severity was mostly minimal, rising to slight and moderate in a few cases. In the F2B offspring, there was no clear test article effect on the incidence of this finding.

In addition, an increased incidence in high dose group kidneys from rats of the F1 generation was observed, in the form of tubular casts in males and pelvic calcinosis in females, both findings chiefly at minimal to slight degrees of severity.

Based on these findings and taking into consideration the fact that only a small number of kidneys were observed at low- and mid- dose in the F1 and F2B animals, it is difficult to conclude that these findings constitute a true adverse effect. A reproduction toxicity study is basically designed to evaluate the potential effects of an active ingredient on the reproductive performance of the treated animals. In this study, although some effects were observed in the kidneys, that were consistent with the observations previously made in other toxicity study conducted with this material, the main conclusion is that these changes did not affect the reproduction parameters.

Therefore, for the setting of an AEL for systemic chronic exposure, one should rather consider a long-term study in which the endpoint of interest has been fully evaluated at all dose levels over a much longer time period and in a significantly greater population of animals.

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BPD Data set IIA/ Annex Point VI.6.9

Neurotoxicity

Acute Oral Rat neurotoxicity Study

Official use only

1 REFERENCE

1.1 Reference

[redacted] (1998).

Bay U 4619 (NAK 4455): CNS-safety pharmacology after a single oral administration in rats (Study P 9011863). [redacted]

[redacted] Report No. PH-27592 [BES Ref: MO-03-009700]

Report date: June 26, 1998

Unpublished

Amendment: [redacted] (1999).

Amendment to Report No. PH27592, Bay U 4619 (NAK 4455): CNS-safety pharmacology after a single oral administration in rats (Study P 9011863). [redacted].

[redacted] Report No. PH-27592A [BES Ref: MO-04-002968]

Report date: August 16, 1999

Unpublished

1.2 Data protection Yes

1.2.1 Data owner: Bayer CropScience

1.2.2 Companies with letters of access:

1.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study This study addresses only the motor activity (open field study) part of OECD TG 424.

2.2 GLP Yes

2.3 Deviations Yes; neurohistology, clinical observations, and a complete functional observational battery are not included.

3 MATERIALS AND METHODS

3.1 Test material BAY U 4619 (transfluthrin)

3.1.1 Lot/Batch number: 816679301

3.1.2 Specification:

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3.1.2.1	Description	Yellowish crystalline compound
3.1.2.2	Purity	95.5%
3.1.2.3	Stability	Analytical data from March 1998 verify that the test material is chemically stable within the concentration range of 1–100 mg/ml in PEG 400; Under current sample preparation and handling conditions, stability was assured at room temperature for at least 6 hours.
3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar (HsdCpb: WU)
3.2.3	Source	[redacted]
3.2.4	Sex	Male
3.2.5	Age/weight at study initiation	Approximately 6 weeks of age, within a weight range of 173-223 g.
3.2.6	Number of animals per group	6 – for combined temperature/catalepsy test 10 – for open field test of psychomotoric activity
3.2.7	Control animals	Yes – concurrent control group given vehicle only
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	Single dose
3.3.2	Frequency of exposure	1 dose
3.3.3	Postexposure period	Up to 4 hours
3.3.4	**Oral**	
3.3.4.1	Type	Gavage
3.3.4.2	Concentration	0, 10, 30, 100 mg/kg in a volume of 5 mL/kg
3.3.4.3	Vehicle	PEG 400
3.3.4.4	Controls	PEG 400
3.4	**Examinations**	
3.4.1	Observations	
3.4.1.1	Clinical signs	No
3.4.1.2	Mortality	No
3.4.2	Body weight	No
3.4.3	Food consumption	No
3.4.4	Water consumption	No

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3.4.5	Ophthalmoscopic examination	No
3.4.6	Haematology	No
3.4.7	Clinical Chemisty	No
3.4.8	Urinalysis	No
3.5	**Sacrifice and pathology**	
3.5.1	Organ Weights	No
3.5.2	Gross and histopathology	No
3.5.3	Other examinations	A body temperature test was performed. Just before dosing the body temperature of each animal was measured electronically with a stomach probe, and again 30, 60, 90, 120, 180, and 240 minutes after dose administration. A test to detect catalepsy was performed. The ability of the animal to withdraw a forepaw from a corkblock was determined. If a rat remained in this unusual position for at least 15 seconds the test was regarded to be positive. Psychomotor activity was determined by an open-field test. At 30, 60, and 120 minutes after administration of the dose, animals were placed singly in open-field boxes (45 x 45 cm) for 5 minutes. Three parameters were measured automatically – travelled distance, resting time, and number of rearings. Details regarding the testing and measuring apparatus were not given.
3.5.4	Statistics	Data from the body temperature test and the open-field test were compared using an ANOVA with repeated measurements amd the Duncan's multiple range test. Level of significance was $p<0.05$. Catalepsy scores of treated animals were compared to scores of the vehicle controls.
3.6	**Further remarks**	
		4 RESULTS AND DISCUSSION
4.1	**Observations**	
4.1.1	**Clinical signs**	After treatment core body temperature increased in all groups including controls. No statistical analysis was performed to determine significance. No other clinical signs were noted.
4.1.2	**Mortality**	None.
4.2	**Body weight/body weight gain**	Not given.
4.3	**Food consumption and compound**	Not given.

	intake	
4.4	**Functional Observational Battery (FOB)**	Not noted
4.5	**Motor Activity**	A test to determine the cataleptic activity of transfluthrin was performed at 30 minute intervals until 240 minutes after administration of the a.s. Catalepsis (as defined by the failure to withdraw within 15 seconds, a paw placed on a cork block) was seen in a few animals of each group but without relationship to treatment. The report concludes this to be vehicle-related. Validity of the paw withdrawal action as a test for catalepsy, is unclear. An open field test was performed to determine travelled distance, resting time, and number of rearings as a measure of acute motor activity after treatment with transfluthrin. Results are given in tables A6.9-1, 2 and 3 below. Overall, transfluthrin had no effect on motor activity. Posthoc comparisons indicated a statistically significant increase in the travelled distance in animals given 100 mg/kg at 60 minutes after treatment. There was a corresponding reduction in resting and increase in rearing for that dose and time point. The rate of adaptation was not differentially affected by the a.s. for distance and resting (Time by Group interactions), but was significant for rearing which decreased progressively over time (Factor by Time interaction), indicating that all animals adapted to the open field regardless of treatment.
4.6	**Sacrifice and pathology**	
4.6.1	Organ weights	Not measured
4.6.2	Neuro-histopathology	Not evaluated
4.7	**Other**	None

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1	**Materials and methods**	Groups of 10 male HsdCpb:WU rats were exposed to one dose of transfluthrin in PEG 400 vehicle by gavage at doses of 0, 10, 30, and 100 mg/kg in a dosage volume of 5 mL/kg. Motor activity was automatically measured in an open field test at 30, 60, and 120 minutes after dosing. The parameters measured were distance travelled, time resting, and number of rearings. This study partially fulfills requirements of OECD 424, Neurotoxicity in Rodents.
5.2	**Results and discussion**	The principal finding was a lack of effect of transfluthrin on motoric activity. In the open field test, the only significant change observed was a decrease in rearing activity over time, and this was due to normal adaptation rather than a treatment effect. This effect was not reflected by similar changes in the adaptation rate for the other parameters tested, travelled distance and resting time. It was driven by the decreased number of rearings in the 30 mg/kg group 30 minutes after dosing, but not later on, and by the relative increase in rearing in the high dose

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		group 60 minutes after dosing, but not at 30 or 120 minutes post-administration. Therefore the decrease in rearing activity in the 30 mg/kg group is considered incidental. It is not seen at higher doses nor is it reflected by significant parallel changes in the other locomotor measurements. The increase in exploratory activity observed in the high-dose group might be treatment-related. However, the effect is quantitatively small in size, and restricted to a single observation time point, suggesting that the effect is not pharmacologically or clinically relevant. Therefore, transfluthrin treatment is not considered to influence the motor activity of rats. Absence of most usually-reported data (including symptoms and bodyweights) has the consequence that some data (e.g. results for catalepsy) cannot be meaningfully interpreted.
5.3	**Conclusion**	
5.3.1	LO(A)EL	No treatment-related effect was seen in this study.
5.3.2	NO(A)EL	The acute NOAEL was determined to be 100 mg/kg based on a lack of effect on motor activity at the highest dose level tested.
5.3.3	Other	Treatment with transfluthrin is not considered to influence the acute motor activity of rats.
5.3.4	Reliability	2. This study adequately reports motor activity during the initial 4 hours post-dosing, so partially fulfils OECD 424 guidelines
5.3.5	Deficiencies	This study lacks neurohistological and clinical data, and an FOB for time periods later than four hours post-dosing.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	15 February 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	see conclusion below
Conclusion	LO(A)EL: Not established NO(A)EL: Not established Other conclusions: The study set up shows many deficiencies and the number of parameters tested is very limited. No individual data have been presented. In view of the very limited number of parameters that has been tested in male animals only, this can not be deemed an adequate neurotoxicity study. Therefore it seems not appropriate to derive a NOAEL or LOAEL from this study. This study is considered supplementary
Reliability	3

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Acceptability	Acceptable but supplementary
Remarks	
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

125.3.4.1.1.1 TABLE A6.9- 1: SUMMARY OF TREATMENT-RELATED FINDINGS, ACUTE MOTOR ACTIVITY DISTANCE TRAVELLED

Group	**Dose of Transfluthrin (mg/kg oral)**	**Number of animals**	**Travelled distance in meters (Mean ± S.D.)**		
			30 min	60 min	120 min
1 (control)	0 (vehicle)	10	13.6 ± 4.12	4.4 ± 2.83	5.6 ± 4.49
2	10	10	13.8 ± 3.00	7.0 ± 4.49	6.7 ± 3.9
3	30	10	10.7 ± 4.14	6.4 ± 4.36	5.0 ± 4.07
4	100	10	13.3 ± 4.52	8.8 ± 4.31*	6.8 ± 4.18
Comments	* = p<0.05 compared to the control group, Duncan's multiple range test				

125.3.4.1.2 Table A6.9- 2: Summary of Treatment-Related Findings, Acute Motor Activity Resting Time

Group	**Dose of Transfluthrin (mg/kg oral)**	**Number of animals**	**Resting time in seconds (Mean ± S.D.)**		
			30 min	60 min	120 min
1 (control)	0 (vehicle)	10	158.6 ± 28.29	245.1 ± 32.87	233.1 ± 42.42
2	10	10	156.4 ± 25.83	221.5 ± 45.98	220.3 ± 38.9
3	30	10	185.4 ± 36.95	224.9 ± 41.12	237.7 ± 40.13
4	100	10	165.9 ± 35.97	203.0 ± 34.85*	220.1 ± 42.76
Comments	* = p<0.05 compared to the control group, Duncan's multiple range test				

125.3.4.1.3 Table A6.9- 3: Summary of Treatment-Related Findings, Acute Motor Activity Rearings

Group	**Dose of Transfluthrin (mg/kg oral)**	**Number of animals**	**Number of rearings (Mean ± S.D.)**		
			30 min	60 min	120 min
1 (control)	0 (vehicle)	10	22.7 ± 8.85	3.9 ± 4.01	4.2 ± 4.64
2	10	10	25.9 ± 9.96	7.5 ± 6.42	7.3 ± 7.85
3	30	10	12.7 ± 8.71*	5.2 ± 4.42	3.7 ± 5.91
4	100	10	19.8 ± 9.32	12.4 ± 11.79*	6.4 ± 5.87
Comments	* = p<0.05 compared to the control group, Duncan's multiple range test				

Doc. IIIA	**Neurotoxicity**
Section A 6.9	Developmental neurotoxicity Study
BPD Data set IIA/ Annex Point VI.6.9	

Doc. IIIA
Section A 6.9
BPD Data set IIA/ Annex Point VI.6.9

Neurotoxicity

Developmental neurotoxicity Study

1.1	**Reference**	[redacted] (2007) A developmental neurotoxicity study with technical grade transfluthrin in Wistar rats [redacted] Report No. 201619 [BES Ref: M-285100-01-1] Report date: February 16, 2007 Unpublished
1.2	**Data protection**	Yes
1.2.1	Data owner	Bayer CropScience
1.2.2	Companies with letter of access	
1.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I
		2. GUIDELINES AND QUALITY ASSURANCE
2.1	**Guideline study**	U.S. EPA, OPPTS 870.6300 OECD TG 426 (draft) Health Canada PMRA DACO No. 4.5.14
2.2	**GLP**	Yes
2.3	**Deviations**	No
		3. MATERIALS AND METHODS
3.1	**Test material**	Transfluthrin technical
3.1.1	Lot/Batch number	EATFTJ005
3.1.2	Specification	
3.1.2.1	Description	Colourless liquid
3.1.2.2	Purity	99.1%
3.1.2.3	Stability	Stable at room temperature

Doc. IIIA **Neurotoxicity**

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3.2	**Test Animals**	
3.2.1	Species	Rat
3.2.2	Strain	Wistar crl:WI(Han)
3.2.3	Source	[redacted]
3.2.4	Sex	Males and females
3.2.5	Age/weight at study initiation	At least 15 (males) and 12 (females) weeks of age at co-housing, within a weight range of 198.6-249.1 g for females, no specified weight requirements for males
3.2.6	Number of animals per group	30 females per group
3.2.7	Control animals	Yes – concurrent control group given control diet
3.2.8	Mating period	5 consecutive days
3.3	**Administration/ Exposure**	
3.3.1	Duration of treatment	Daily, starting on Gestation Day (GD) 6 and continuing for the dams and offspring until lactation day (LD) 21
3.3.2	Postexposure period	None
3.3.3	Exposure	Oral
3.3.3.1	Type	Dietary
3.3.3.2	Concentration	0, 500, 2000, 7000 ppm with adjustments during lactation to maintain a more constant dosage throughout exposure
3.3.3.3	Vehicle	None
3.4	**Examinations**	

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3.4.1	Parental generation		
3.4.1.1	Clinical observations	Following acclimation and continuing until animals were removed from the study, P-generation males and females were observed (cage-side) for clinical signs at least once daily.	
3.4.1.2	Detailed observation	A detailed evaluation of the dams for clinical signs with a physical examination was conducted once daily from the initiation of exposure (GD 6) through lactation day 21.	
3.4.1.3	Functional Observational Battery (FOB)	Animals that were presumed to be pregnant (approximately 30 per dietary level) were observed on GD 13 and GD 20 and a minimum 10 dams/dietary level that were maintained on study with suitable litters were also observed on LD 11 and LD 21. This observational battery included, but was not limited to, assessments (with severity scoring) of lacrimation, salivation, piloerection, exophthalmia, urination, defecation, pupillary function, palpebral closure, convulsions, tremor, abnormal movements, unusual behaviours, posture and gait abnormalities.	
3.4.1.4	Body weight and food consumption	Body weight and food consumption were measured once weekly during gestation and lactation, as follows: GD 6-13, 13-20 and LD 0-7, 7-14 and 14-21. In addition, dams were weighed on LD 4. Measures of food consumption may have included consumption by the pups, especially during the third week of lactation.	
3.4.1.5	Delivery and culling	Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated lactation day 0 (LD 0) for the dam and postnatal day 0 (PND 0) for the pups. Litter size (the number of pups delivered) and pup "status" at birth were recorded for each litter.	
3.4.2	F1 generation		
3.4.2.1	Litter observations	As soon as possible following parturition, pups were examined for ano-genital distance to establish their gender, and then were tattooed and weighed. Live pups were counted, sexed and weighed individually for each litter on PND 0, 4, 11, 17, and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality or morbidity. Any gross signs of toxicity in the offspring were recorded as they were observed, including the time of onset, degree, and duration. More detailed observations for clinical signs were made once daily (a.m.) before weaning and once weekly thereafter. After weaning on PND 21, the remaining pups were weighed once weekly, as well as when vaginal patency or balanopreputial separation was first evident, with detailed observations for clinical signs performed once weekly.	

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Section A 6.9 Developmental neurotoxicity Study

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3.4.2.2	Developmental landmarks (sexual maturation) and pupil constriction	Beginning on PND 38, male offspring were examined daily for balanopreputial separation. Beginning on PND 29, female offspring were examined daily for vaginal patency. The age of onset was recorded. On PND 21, all pups were also tested for the presence of pupil constriction.	
3.4.2.3	Post weaning observation	After weaning on PND 21, offspring were examined twice daily for mortality, and cage-side observations were conducted once daily. Individual offspring body weight data were recorded weekly, as well as on the day that vaginal patency or balanopreputial separation was achieved.	
3.4.2.4	Body weight and food consumption	Surviving pups were weighed on PND 0, 4, 11, 17, and 21, and once weekly thereafter. The individual pups were also weighed when vaginal patency or balanopreputial separation were first evident. Food consumption was not measured after weaning on PND 21, when all animals received untreated diet.	
3.4.2.5	Neurobehavioral evaluations		
3.4.2.5.1	Functional Observational Battery (FOB)	On PND 4, 11, 21, 35(± 1d), 45(± 1d), and 60 (± 2d), approximately 20 offspring/sex/group (representing at least 20 litters per level) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage involved. This evaluation was performed according to the procedures described for maternal animals, using standardized procedures. The only difference is that the neonates (i.e., PND 4 and 11) were not evaluated in the open field, since this is routinely done only if the observer considers it necessary for evaluation and this was not the case in the present study.	
3.4.2.5.2	Motor activity testing	Motor activity was measured in approximately 20 rats/sex/dose (representing at least 20 litters/dietary level) on PND 13, 17, 21 and 60 (± 2d). The same offspring were evaluated in the figure-eight maze for 60 minutes at each time point.	
3.4.2.5.3	Auditory startle reflex habituation	Auditory startle reflex habituation testing was performed in approximately 20 rats/sex/dose (representing at least 20 litters/dose) on postnatal days 23 and [60 (±2 days)], using an automated system.	
3.4.2.5.4	Learning and memory testing	<u>Post-weaning passive avoidance:</u> Animals were tested for acquisition on PND 23 and for retention on PND 30. <u>Adult (PND 60) Offspring – water maze:</u> the animals assigned to passive avoidance testing were also assigned to water maze testing. Animals were tested on postnatal day 60 (± 2 days), and again seven days later. Only animals that demonstrated acquisition were tested for retention. The water in the M-maze was maintained at 22 +1°C.	

3.4.2.6 Ophthalmology	At approximately 50-60 days of age, ophthalmic exams were conducted using the males and females (a minimum of 10/sex/dietary level; representing at least 20 litters per level) that were selected for perfusion at study termination.	
3.4.3 Post-mortem observations		
3.4.3.1 Maternal animals	Maternal animals were sacrificed by carbon dioxide (CO_2) asphyxiation on day 21 of lactation following the weaning of their respective litters. The dams were discarded without post-mortem examination. Females that were sperm positive and/or had an internal vaginal plug but did not deliver were sacrificed on GD 24 without necropsy examination.	

3.4.3.2	Offspring	Necropsy: the offspring, selected for brain weight or neuropathological evaluation, were sacrificed on PND 21 or 75 (+5 days). F1-generation animals that were found moribund (if any) while on study were sacrificed and underwent a gross necropsy examination. Tissues were collected at the discretion of the study director. In addition, randomly-selected animals (neurobehavioral groups) that were used to measure fresh brain weight underwent a necropsy examination. Where required, the necropsy involved an examination of all organs (including the brain), body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Other gross lesions were generally not collected for microscopic examination. Animals found dead (if any) underwent a necropsy examination and were disposed of without the routine collection of tissues. Perfusion: animals that were selected for perfusion on PND 21(from Set D) or at study termination (from Set A-C) were deeply anesthetized and then perfused via the left ventricle. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves) and selected (bilateral) peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. Measurements: - anterior-to-posterior (AP) length of the cerebrum, extending from the anterior pole to the posterior pole, exclusive of the olfactory bulbs; - anterior-to-posterior (AP) length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. Histology: the brain tissue from perfused animals and any gross lesions collected at necropsy were further processed for microscopic examination. The brain was divided into 8 coronal sections. Aditionnal tissues were collected for microscopic examination from perfused animals including 3 levels of spinal cord (cervical, thoracic and lumbar), the cauda equine, eyes, optic nerves and gastrocnemius muscle. Micropathology and morphometry: the tissues from high-dose animals were examined relative to those from the respective control group. If no treatment-related lesion were evident further analysis was not performed.	
		4. RESULTS AND DISCUSSION	
4.1	**Parental animals**		
4.1.1	Clinical signs	Compound-related clinical signs were not evident at any dietary level. One finding that was considered incidental and unrelated to treatment was areas of hair loss (alopecia) for 2 low- and 1 high-dose females. This is a common finding during gestation that is associated with nest-building behavior in pregnant rat.	
4.1.2	Mortality	No P-generation females were found dead during gestation or lactation. There were also no P-generation males found moribund or dead after initiation of the study (males only received untreated diet).	
4.1.3	Functional Observational Battery (FOB)	Compound-related findings were not evident at any dietary level.	

4.1.4	Body weight and food consumption	See Table 6.9-1 Gestation: With start of treatment, food consumption was statistically increased (+86%) at the highest dose compared to controls but not at the lower dose levels. This was associated with excessive feed spillage on GD6-13. Thereafter type of feeders was changed for high dose dams to avoid spillage with the consequence that food consumption was not different from control on GD 13-20 at any dietary level. Bodyweight was not affected by treatment at any dose level although bodyweight gain was reduced 10% form GD0-20 for high dose dams compared to controls. This difference was attributed to palatability which was evident at this dose level as shown by feed spillage. Lactation: During the first week of lactation, food consumption was increased for low- and mid-dose dams (statistically +29% and non-statistically +23%, respectively). This was associated with excessive feed spillage. After change of feeders to limit spillage, food consumption was not different from controls thereafter. On LD0, 4 and 7, bodyweight was statistically reduced (5-6%) in high dose dams, compared to controls, reflecting the lower bodyweight gain observed during gestation. Bodyweight were comparable top controls at all dietary levels on LD14 and 21. Overall the differences from controls (increased food wastage at all dietary levels and decreased bodyweight and bodyweight gain at the highest dose level) were thought to be related to palatability.	
4.1.5	Test substance intake	Table A.6.9-2 The average daily intake of transfluthrin was calculated using weekly bodyweight and food consumption data. The average daily intake of transfluthrin during gestation and lactation was 0, 42.1, 161 and 531 mg/kg bw/d.	
4.1.6	Reproductive performance	Reproduction parameters were not affected by the test substance at any dietary level. The fertility index at the high dose level was 86.7% compared to 100% for control and 93.3% for low- and mid-dose animals however this value was within the historical control range (83.3-100%).	
4.2	**Offsprings (F1 generation)**		
4.2.1	Viability and clinical signs	Litter parameters and pup viability were not affected by treatment at any dietary level. There were no compound-related signs during lactation in males or females at any dietary level. There were no compound-related clinical signs after weaning (when exposure was discontinued) in males or females at any dietary level.	

4.2.2 Body weight	Table A 6.9-3 Preweaning: There was no difference in birth weight or PND4 bodyweight, in either sex. Bodyweight was 9% lower on PND11 in the high-dose females but bodyweight was not affected at any dietary level at any other time-point. Bodyweight gain was statistically decreased on PND 4-11 and PND 4-21 in high dose males and females (-8 to -11%). The statistical difference from control in low dose females on PND 4-11 was considered incidental and not related to treatment since it was modest, only seen in one sex, not seen in either sex at the mid dose and bodyweight was not affected. Post-weaning: after weaning and discontinuation of treatment, bodyweight was not different from controls at any dietary level, in either sex.	
4.2.3 Developmental landmarks (sexual maturation) and pupil constriction	The age for onset of balanopreputial separation, of vaginal patency and for surface righting were not affected by treatment at any dose level. Pupil constriction in response to a penlight was apparent in all pups on PND 21.	
4.2.4 Behavioural assessments		
4.2.4.1 Functional Observational Battery (FOB)	There were no treatment-related findings in either sex at any dietary level.	
4.2.4.2 Motor and locomotor activity	There were no compound-related effects on measures of motor or locomotor activity in males or females at any dietary level. Moreover, there were no statistical differences from control at any dose level on any test occasion. A comparison of interval results for control and treated animals revealed no compound-related effects at any dietary level. Levels of motor and locomotor activity were generally comparable to control for all test intervals on all test occasions. Moreover, there were no statistical differences from control in males or females at any dietary level on any test occasion.	
4.2.4.3 Auditory startle habituation	Startle amplitude, latency and habituation were not affected by treatment at any dietary level, on any test occasion. There were a few statistical differences from control for mid- and high-dose females on PND 23 and PND 60. None of these differences from controls were considered treatment-related since there was no relationship to dose, they were only seen in one sex, habituation was not affected and the findings were inconsistent (i.e. decreased response amplitude on PND 23 vs. an increase on PND 60)	
4.2.4.4 Learning and memory testing	Post-weaning – Passive avoidance: for acquisition and retention, there was no evidence of a compound-related effect in males or females at any dietary level. Moreover, there were no statistical differences from control at any dietary level in either sex. Adult (PND 60) offspring – Water maze: there were no compound-related effects in males or females at any dietary level. Furthermore, there were no statistical differences from control at any dietary level on either test occasion.	

4.2.5 Ophthalmology	There were no compound-related lesions in males or females at any dietary level.	
4.2.6 Post-mortem results	Gross pathology: there were no compound-related necropsy findings in animals that were either found dead or sacrificed on PND 21 or at study termination. Bodyweight: *Day 21* - Terminal body weight for for perfused male and female was not affected by treatment. *Terminal* – terminal body weight for perfused males and females and non-perfused females was not affected by treatment at any dietary level. Brain weight: *Day 21* - Absolute and relative fixed brain weights were not affected by treatment in males or females at any dietary level. *Terminal* - Absolute and relative fixed brain weights for terminal perfused males and females and non-perfused terminal females were not affected by treatment at any dietary level. Absolute brain weight for non-perfused terminal males was also unaffected by treatment. Relative brain weight for low-dose, non-perfused males was statistically increased due to decreased terminal bodyweight Brain measurement morphometry: Table A 6.9-4 *Day 21 pup gross brain measurements*: there were no treatment-related significant differences in gross necropsy cerebrum or cerebellum length in males and females at any dietary level. *Terminal animal gross brain measurements*: for perfused terminal adults, the cerebrum and cerebellum lengths were comparable to control for males and females at all dietary levels. *Day 21 pup micropathology brain measurements*: there were no statistically significant differences in micropathology brain measurements in high dose males. In high- and low-dose females, the hippocampus thickness was less than controls but not in mid-dose females. These differences were judged incidental and not related to treatment based on the absence of dose-relationship (-14% for low dose vs. -5% for the high dose) and the consistency with historical control values. *Terminal animal micropathology brain measurements*: there were no compound-related effects on any brain measurement in high-dose males or females. The decreased hippocampus measurement relative to controls was not considered to be biologically relevant since it was modest (-8%). The increased frontal cortex thickness observed at all dietary levels compared to controls was not attributed to treatment since there was no dose-relationship and the control value was below the range of historical controls whereas values at all dietary levels were comparable to historical control.	

	Micropathology: *Day 21 pup micropathology*: there were no compound-related microscopic findings in brain tissues from perfused PND 21 high-dose males or females. *Terminal animal micropathology*: there were no compound-related microscopic findings in brain tissues from perfused terminal high-dose males or females. The only lesion (optic nerve atrophy) was observed in the brain on 1 control female. Additional non-brain terminal animal tissues: there were no compound-related microscopic lesions present in any tissue from the high-dose males or females.	
	5. Applicant's Summary and conclusion	
5.1 Materials and methods	The principal objective of this developmental neurotoxicity study was to investigate the potential for technical grade transfluthrin to produce functional and morphological effects on the nervous system of offspring from oral (dietary) exposure during pregnancy and lactation. Technical grade transfluthrin was administered via the diet from gestation day (GD) 6 through lactation day (LD) 21 to mated female Wistar rats at nominal concentrations of 0, 500, 2000 and 7000 ppm, with adjustments during lactation to maintain a more consistent dosage throughout exposure. All test diets (including control) were provided for *ad libitum* consumption throughout the study, except during neurobehavioral testing. Concentration in the diet, as well as the homogeneity and stability of deltamethrin in the dietary ration, was confirmed. On postnatal day (PND) 4, litters with a minimum of seven pups, including at least three per sex, were culled to yield, as closely as possible, four males and four females. Subsets of surviving offspring, representing at least 19-20 litters per dietary level, were subjected to evaluation using the following observations and measurements: detailed clinical observations and a functional observational battery, preputial separation or vaginal patency, body weight, automated measures of activity (figure-eight maze), auditory startle habituation, learning and memory (passive avoidance after weaning and a water maze task beginning on PND 60±2 days) and an ophthalmic examination. Neural tissues were collected from 10/sex/dietary level (representing 20 litters) on PND 21 (brain only) and at study termination (approximately 75 days of age) for microscopic examination and morphometry.	
5.2 Results and discussion	The mean daily intake of the test substance (mg transfluthrin/kg bw/day) based on the average dietary consumption for the last two weeks of gestation and three weeks of lactation at nominal dietary concentrations of 20, 80 or 200 ppm, respectively, was 0, 42.1, 161 and 534 mg/kg bw/day.	X

	Treatment-related effects attributed to exposure to transfluthrin were as follows: **<u>Maternal</u>** 500 ppm - There were no treatment-related findings during gestation or lactation. 2000 ppm - There were no treatment-related findings during gestation or lactation. 7000 ppm - There were no treatment-related findings during gestation or lactation. Bodyweight gain during gestation was reduced 10% compared to controls and bodyweight was statistically reduced (6% maximum) on LD0, 4 and 7. These differences from control were ascribed to palatability and were not considered an adverse effect. Thus, the maternal NOAEL is 534 mg/kg bw/day. **<u>Offspring</u>** 500 ppm - There were no treatment-related findings. 2000 ppm - There were no treatment-related findings. 7000 ppm - Bodyweight was statistically decreased (9%) in females on PND 11. Bodyweight gain was statistically decreased on PND 4-11 in females and combined males and females (11% and 10%, respectively). Also, bodyweight gain was statistically decreased 8-9% on PND 4-21 in males and females. Thus, the offspring NOAEL is 161 mg/kg bw/day, based on decreased bodyweight in PND 11 females, reduced bodyweight gain on PND 4-11 in females and in combined males and females and on PND 4-21 in both sexes and combined sex that were observed at 534 mg/kg bw/d (= offspring LOEL). These effects at the highest dose level were associated with decreased bodyweight in the dams, compared to controls.	
5.3 **Conclusion**	Transfluthrin is not a developmental neurotoxicant when administered at the highest tolerated dose (7000 ppm = 534 mg/kg bw/day) to pregnant rats from GD6 to LD 21.	
5.3.1 Reliability	1	
5.3.2 Deficiencies	None	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7 June 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	5.2. the nominal dietary concentrations should be 500, 2000 and 7000 ppm.. Otherwise the version of the applicant is adopted.

Conclusion	LO(A)EL: 7000 ppm, equal to 530 mg/kg bw/day (based on the mean maternal substance intake from PND 0-14), on the basis of decreased body weight (9%) in female pups on PND 11 and bodyweight gain (10%) during PND 4-11 for male and female pups combined. NO(A)EL: 2000 ppm, equal to 166 mg/kg bw/day (i.e. mean maternal substance intake from PND 0-14) Other conclusions: Transfluthrin is not a developmental neurotoxicant at doses up to and including 7000ppm when administered to pregnant rats from GD6 to LD 21.
Reliability	1
Acceptability	acceptable
Remarks	The levels of transfluthrin in milk were not measured. Thus it is not clear if and at which level the pups were exposed to transfluthrin after birth. In an inhalation study in mice, performed by Ivens et al (1996), effects on brain muscarinergic receptor levels were found. In the present study the levels of muscarinergic receptors in the brain were not measured. I In a position paper dated 25/6/2008 the applicant described that the detection of transfluthrin in the offspring liver and kidney, on lactation days 10, 14, 18 and 21 provides evidence that the pups were exposed to the active ingredient via milk with higher levels at the end of lactation when pups were exposed via both the treated diet and the milk thus supporting dietary administration in the main DNT study. However, the RMS still has doubts, whether the offspring was exposed enough during the development of the brain. 31-3-2011: The increases in muscarinic receptor levels in the cortex of the brain is not biological relevant.. The measurement of this parameter is not a requirement in U.S. EPA, OPPTS 870.6300, OECD Test Guideline 426 (Draft) or Health Canada PMRA DACO No. 4.5.14. Moreover, it has not been established whether this parameter in mice is of clinical significance in humans. There was no correlation reported between transitory increase in muscarinic receptor densities (PND17, but no change apparent at 4 months) with motor activity. A transfluthrin DNT pilot study (described in page 20-21 of Gilmore et al, 2007) was conducted prior to the main study to determine how Wistar rats would tolerate exposure to a dietary concentration of 5000 ppm from gestation day (GD) 6 through day 21 of lactation. The pilot DNT study was designed to verify the exposure of the offspring during lactation by measurement of transfluthrin in pup tissue. In this study, eight pregnant Wistar rats were treated via the diet to a nominal concentration of 5000 ppm transfluthrin from gestation day 6 through lactation day 21, with adjustments in dietary level during lactation to maintain a more constant dosage (mg/kg/day) throughout exposure. Offspring from each litter were sacrificed on lactation days 10, 14 and 16 to measure the concentration of transfluthrin in the brain. There were no compound-related effects apparent in the dams or offspring, although the small sample size and lack of concurrent control group limits the ability to identify relatively subtle effects (e.g., a slight decrease in weight

	gain). There were no detectible residues in the brain on post-natal day (PND) 10 and very lowresidues on PND 16.Based on these results, another pilot study was conducted to test whether a higher dietary level of 7000 ppm transfluthrin would be suitable for a DNT study and to determine whether residuescould be detected in liver and kidney tissues to provide evidence of pup exposure to transfluthrin during lactation. These tissues were selected for analysis based on evidence in the ADME study that they contain relatively higher concentrations of transfluthrin than other tissues, including brain (Minor and Freeseman, 1991). In this second pilot study (described in page 21 of Gilmore et al, 2007), six pregnant Wistar rats were exposed to a nominal concentration of 7000 ppm transfluthrin in the diet from gestation day 7 through lactation day 21, with adjustments in dietary levels during lactation to maintain a more constant dosage (mg/kg/day) throughout exposure. Offspring from each litter were sacrificed on lactation days (LD) 10, 14, 18 and 21 (one/sex/litter at each age) to measure the concentration of transfluthrin in the liver and kidney. Compound-related effects included tremor for three out of six dams, beginning on lactation day 3 in two animals and lasting up to five days. The results of this study revealed residues of transfluthrin in pups at all ages. These results clearly demonstrate that the offspring were exposed to transfluthrin mixed with the rodent diet during lactation, with higher levels at the end of lactation when pups were exposed via both the treated diet and the milk thus supporting dietary administration in the main DNT study. The 7000 ppm dietary level was selected as a maximum dose the animals would tolerate without excessive toxicity. In the subsequent main DNT study conducted with transfluthrin, no effects were observed in the dams nor in the offspring up to and including 2000 ppm equivalent to 161 mg/kg bw/day. Effects were confined to the high dose level (7000 ppm equivalent to 534 mg/kg bw/day) and included a 10% decrease in body weight gain during gestation and a decrease in body weight up to 6% on LD 0, 4 and 7 in dams. However, these effects were ascribed to palatability and were considered as not adverse. In the offspring, body weight was statistically decreased (9%) in females on PND 11 and statistically decreased on PND 4-11 in both females and males (11% and 10%, respectively). Body weight was also significantly statistically decreased by 8-9% on PND 4-21 in both sexes. The No Observed Effect Level (NOEL) for both dams and the offspring was 2000 ppm equivalent to 161 mg/kg bw/day based on the bodyweight effects reported at 7000 ppm.	
	COMMENTS FROM ... *(specify)*	
Date	*Give date of comments submitted*	
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion. Discuss if deviating from view of rapporteur member state*	
Results and discussion	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Reliability	*Discuss if deviating from view of rapporteur member state*	
Acceptability	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Table A6.9- 1: Mean (±SD) Maternal Body Weight and Food Consumption [a]

Observations/study week	Dose (PPM in diet)			
	Control	**500 PPM**	**2000 PPM**	**7000 PPM**
Gestation				
Mean body weight (g) Gestation day 0	231.9±2.34 (30)	226.2±1.75 (28)	230.3±2.33 (27)	228.4±2.63 (26)
Mean body weight (g) Gestation day 6	251.5±3.06 (30)	244.5±1.51 (28)	246.6±3.97 (27)	248.4±2.85 (26)
Mean body weight (g) Gestation day 13	276.4±3.72 (30)	269.0±2.10 (28)	273.8±3.28 (27)	267.7±3.09 (26)
Mean body weight (g) Gestation day 20	337.1±5.28 (30)	328.3±2.95 (28)	330.1±4.39 (27)	323.2±3.45 (26)
Mean weight gain (g) Gestation days 0 - 20	105.2±3.94 (30)	102.1±2.53 (28)	99.8±2.96 (27)	94.8±2.22 (26)
Mean food consumption (g/animal/day) Gestation days 6 - 13	22.7±0.78 (30)	22.0±0.77 (27)	20.9±0.59 (26)	**42.3**±4.67[1] (26)**
Mean food consumption (g/animal/day) Gestation days 13 - 20	23.5±0.71 (30)	23.9±0.80 (28)	22.5±0.66 (26)	22.3±0.81 (25)
Lactation				
Mean body weight (g) Lactation day 0	267.2±3.98 (30)	260.5±3.03 (28)	265.3±3.50 (27)	**253.5*±3.14 (26)**
Mean body weight (g) Lactation day 4	284.0±4.31 (23)	272.5±3.27 (21)	277.6±4.39 (20)	**267.3*±3.91 (23)**
Mean body weight (g) Lactation day 7	293.3±4.11 (22)	282.1±2.69 (21)	285.1±4.14 (20)	**275.4**±3.58 (23)**
Mean body weight (g) Lactation day 14	305.4±3.62 (22)	296.7±3.31 (21)	301.3±3.90 (20)	292.6±3.65 (23)
Mean body weight (g) Lactation day 21	297.3±3.60 (22)	290.2±3.09 (21)	291.0±4.63 (20)	287.2±3.79 (23)
Mean food consumption (g/animal/day) Lactation days 0 - 7	36.4±1.32 (21)	**47.0*±3.32[1] (21)**	44.7±5.15[1] (19)	36.0±1.20 (23)
Mean food consumption (g/animal/day) Lactation days 7 - 14	53.4±0.86 (22)	52.3±1.03 (21)	51.9±1.08 (20)	51.0±0.83 (23)
Mean food consumption (g/animal/day) Lactation days 14 - 21	62.6±1.04 (22)	61.5±1.07 (20)	59.7±1.26 (20)	60.5±0.85 (23)

[a] *Values are mean ± standard error (n). Means for gestation period include only dams known to deliver pups (either alive or dead).*

* *Statistically different from control, p□0.05*

** *Statistically different from control, p□0.01.*

[1] *associated with observed food spillage*

Table A6.9- 2: **Mean Maternal Test Substance Intake (mg/kg body weight/day)[1]**

Period	Dose (PPM in diet)		
	500 PPM	**2000 PPM**	**7000 PPM**
Gestation			
Gestation days 6 – 13	44.8±1.64 (27)	170.8±5.36 (26)	1179.0±128.14 [2] (26)
Gestation days 13 - 20	44.0±1.41 (28)	164.6±4.19 (26)	582.9±19.04 (25)
Lactation			
Lactation days 0 - 7	46.4±3.30 (21)	173.6±20.08 (19)	516.2±16.2 (23)
Lactation days 7 - 14	39.7±0.80 (21)	157.5±2.95 (20)	543.0±5.7 (23)
Lactation days 14 - 21	35.7±0.65 (20)	139.5±3.16 (20)	494.1±5.71 (23)

[1] *Data obtained from pages 81-82 in the study report. Values are mean ± standard error (n). Dietary concentrations were reduced during weeks 1-3 of lactation (by factors of 1.9, 2.3 and 2.8, respectively), based on estimated increases in feed consumption (g consumed/kg body wt./day) during lactation).*

[2] *Associated with observed food spillage and considered an unreliable measure of a.i. intake. This value was excluded from the mean average daily intake.*

Table A6.9- 3: Pup bodyweight and bodyweight gain

Observations/study week		Dose (PPM in diet)			
		Control	**500 PPM**	**2000 PPM**	**7000 PPM**
Bodyweight (g)					
Mean body weight Day 0	Males	5..9 ± 0.09	5.9 ± 0.11	6.0 ± 0.08	5.8 ± 0.11
	Females	5.6 ± 0.10	5.6 ± 0.11	5.7 ± 0.09	5.5 ± 0.11
Mean body weight Day 4[b]	Males	9.8 ± 0.26	9.7 ± 0.34	10.1 ± 0.21	9.3 ± 0.24
	Females	9.5 ± 0.29	9.3 ± 0.29	9.8 ± 0.21	9.0 ± 0.22
Mean body weight Day 4[c]	Males	9.8 ± 0.25	9.7 ± 0.33	10.1 ± 0.20	9.3 ± 0.25
	Females	9.5 ± 0.30	9.3 ± 0.31	9.8 ± 0.22	9.0 ± 0.23
Mean body weight Day 11	Males	25.4 ± 0.51	24.4 ± 0.76	25.0 ± 0.60	23.6 ± 0.45
	Females	25.1 ± 0.57	23.4 ± 0.70	24.6 ± 0.66	**22.9 ± 0.42***
Mean body weight Day 17	Males	39.7 ± 0.71	37.6 ± 0.97	38.5 ± 0.85	37.0 ± 0.62
	Females	38.6 ± 0.68	36.9 ± 0.95	37.4 ± 0.84	36.1 ± 0.44
Mean body weight Day 21	Males	50.3 ± 0.99	48.1 ± 1.34	48.7 ± 1.13	46.3 ± 0.74
	Females	48.8 ± 0.98	46.4 ± 1.18	47.3 ± 1.17	45.2 ± 0.65
Mean body weight Day 70	Males	339.9 ± 26.2	323.5 ± 24.8	327.5 ± 20.6	325.5 ± 19.9
	Females	204.2 ± 13.1	196.9 ± 12.6	200.8 ± 13.3	199.3 ± 13.5
Bodyweight gain (g)					
Mean body weight gain Day 0-4	Males	3.9 ± 0.21	3.8 ± 0.26	4.1 ± 0.19	3.6 ± 0.16
	Females	3.9 ± 0.22	3.7 ± 0.23	4.1 ± 0.19	3.5 ± 0.15
	Combined	3.9 ± 0.21	3.8 ± 0.24	4.1 ± 0.18	3.5 ± 0.15
Mean body weight gain Day 4-11	Males	15.6 ± 0.31	14.6 ± 0.49	14.9 ± 0.43	14.3 ± 0.29
	Females	15.6 ± 0.32	**14.2 ± 0.44***	14.8 ± 0.50	**13.9 ± 0.25***
	Combined	15.6 ± 0.31	14.4 ± 0.46	14.8 ± 0.45	**14.1 ± 0.26***
Mean body weight gain Day 4-17	Males	29.9 ± 0.58	27.9 ± 0.72	28.4 ± 0.72	27.7 ± 0.51
	Females	29.1 ± 0.48	27.6 ± 0.73	27.6 ± 0.71	27.1 ± 0.36
	Combined	29.5 ± 0.53	27.7 ± 0.72	28.0 ± 0.71	27.4 ± 0.43
Mean body weight gain Day 4-21	Males	40.5 ± 0.83	38.4 ± 1.05	38.5 ± 0.97	**37.0 ± 0.59****
	Females	39.3 ± 0.78	37.2 ± 0.92	37.5 ± 0.99	**36.2 ± 0.51****
	Combined	39.9 ± 0.80	37.8 ± 0.98	38.0 ± 0.95	**36.6 ± 0.53****
Mean body weight gain Day 11-17	Males	14.2 ± 0.36	13.2 ± 0.29	13.5 ± 0.46	13.4 ± 0.27
	Females	13.5 ± 0.24	13.4 ± 0.39	12.8 ± 0.32	13.1 ± 0.23
	Combined	13.9 ± 0.29	13.3 ± 0.32	13.1 ± 0.36	13.3 ± 0.23
Mean body weight gain Day 11-21	Males	24.9 ± 0.56	23.7 ± 0.61	23.6 ± 0.72	22.7 ± 0.36
	Females	23.7 ± 0.53	23.0 ± 0.54	22.7 ± 0.71	22.2 ± 0.32
	Combined	24.3 ± 0.53	23.4 ± 0.56	23.2 ± 0.71	22.5 ± 0.31
Mean body weight gain Day 17-21	Males	10.6 ± 0.42	10.5 ± 0.48	10.1 ± 0.44	9.3 ± 0.33
	Females	10.2 ± 0.37	9.6 ± 0.37	10.0 ± 0.49	9.1 ± 0.35
	Combined	10.4 ± 0.36	10.1 ± 0.40	10.1 ± 0.43	9.2 ± 0.30

[a] *Values are mean ± standard error (n). Means for gestation period include only dams known to deliver pups*

[b] *Before culling*

[c] *After culling*

Table A6.9- 4: Pup brain gross measurement and histopathology findings (females)

Observations/study week	Dose (PPM in diet)			
	Control	**500 PPM**	**2000 PPM**	**7000 PPM**
Gross measurements				
PND 21				
Ant/post cerebrum length (mm)	13.58 ± 0.37	13.60 ± 0.17	13.47 ± 0.28	13.48 ± 0.27
Ant/post cerebrum length (mm)	7.28 ± 0.09	**6.86 ± 0.38***	7.20 ± 0.29	7.04 ± 0.25
PND 75 (±5) (Termination – Perfused)				
Ant/post cerebrum length (mm)	14.90 ± 0.31	14.61 ± 0.25	14.67 ± 0.28	14.73 ± 0.45
Ant/post cerebrum length (mm)	7.79 ± 0.36	7.78 ± 0.40	7.66 ± 0.42	7.64 ± 0.32
Microscopic measurements				
PND 21				
Frontal cortex (mm)	1.706 ± 0.007	--	--	1.722 ± 0.007
Parietal cortex (mm)	1.789 ± 0.007	--	--	1.805 ± 0.005
Caudate Putamen (mm)	2.876 ± 0.011	--	--	2.852 ± 0.008
Hippocampal Gyrus (mm)	1.596 ± 0.004	1.374 ± 0.013	1.509 ± 0.016	1.517 ± 0.003
Cerebellum (mm)	4.596 ± 0.082	--	--	4.609 ± 0.077
PND 75 (±5) (Termination – Perfused)				
Frontal cortex (mm)	1.578 ± 0.003	1.665 ± 0.001	1.659 ± 0.005	1.638 ± 0.005
Parietal cortex (mm)	1.754 ± 0.004	--	--	1.732 ± 0.003
Caudate Putamen (mm)	3.157 ± 0.003	--	--	3.200 ± 0.020
Hippocampal Gyrus (mm)	1.561 ± 0.008	1.598 ± 0.011	1.481 ± 0.013	1.428 ± 0.014
Cerebellum (mm)	4.517 ± 0.055	--	--	4.468 ± 0.067

Values are mean ± standard deviation

** statistically different from control, $p \leq 0.05$*

-- Not evaluated

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126 REFERENCE

3.3	**Reference**	[redacted] (2002). Special toxicity study in female rats for the determination of transitional cell proliferation in the urinary bladder. Dietary administration for 1 and 4 weeks. [redacted]. [redacted] Report No. T 4071411 [BES Ref: MO-04-007453] Report date: November 29, 2002. Unpublished.
3.4	**Data protection**	Yes
3.4.2	Data owner	Bayer CropScience
3.4.3	Companies with letters of access	
3.4.4	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I

4 GUIDELINES AND QUALITY ASSURANCE

4.3	**Guideline study**	No The purpose of the study was to examine cell proliferation in the urinary system of the rat after high dose treatment of the a.s. for up to 4 weeks. There are no applicable guidelines for this specific purpose. The study was well controlled and well conducted with enough animals for good statistical analysis, and is therefore an acceptable study.
4.4	**GLP**	Yes
4.5	**Deviations**	Not applicable

5 MATERIALS AND METHODS

5.3	**Test material**	As given in sections 2 and 3
5.3.2	Lot/Batch number	Batch no. 8169 79301
5.3.3	Specification	As given in sections 2 and 3
5.3.3.1	Description	Yellow to brown, liquid to solidified melt
5.3.3.2	Purity	95.8%
5.3.3.3	Stability	The active ingredient was stable in the feed mixture over a period of 10 days.
5.4	**Test Animals**	
5.4.2	Species	Rat

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5.4.3	Strain	Wistar HsdCpb:wu (historically used at Bayer, so good data on spontaneous alterations)
5.4.4	Source	[redacted]
5.4.5	Sex	female
5.4.6	Age/weight at study initiation	13-14 weeks, mean weight 218.5g (206-243g)
5.4.7	Number of animals per group	Negative control, 1 week – 20 Negative control, 4 weeks – 30 NAK 4455, 1 week – 20 NAK 4455, 4 weeks – 30 Positive control, 1 week – 10 Positive control, 4 weeks - 10
5.4.7.1	at interim sacrifice	At one week – 10 positive controls, 20 negative controls, 20 treated animals
5.4.7.2	at terminal sacrifice	At 4 weeks – 10 positive controls, 30 negative controls, 30 treated animals
5.4.8	Control animals	Yes
5.5	**Administration/ Exposure**	
5.5.2	Duration of treatment	1 or 4 weeks
5.5.3	Interim sacrifice(s)	After 1 week
5.5.4	Final sacrifice	After 4 weeks
5.5.5	Freqency of exposure	Daily
5.5.6	Postexposure period	None
		Oral
5.5.7	Type	In food
5.5.8	Concentration	5000 ppm, food, 327 mg/kg bw/day food consumption ad libitum
5.5.9	Vehicle	Dissolved in peanut oil before being mixed into food. Final: 5000 ppm NAK 4455 in Altromin 1321 diet with 1% peanut oil.
5.5.10	Controls	Negative control - Altromin 1321 diet with 1% peanut oil Positive control – 100 ppm sodium cacodylic acid (NaC) in Altromin diet

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5.6	**Examinations**	
5.6.2	Body weight	Yes
5.6.3	Food consumption	Yes
5.6.4	Water consumption	Yes
5.6.5	Clinical signs	Yes
5.6.6	Clinical Chemistry	No
5.6.7	Urinalysis	Yes Number of animals: All animals Time points: After 2 or 5 weeks of treatment Parameters: Volume, specific gravity, total protein, Na, K, Ca, Cl, tetraflurorbenzoic acid (NAK 4455 metabolite)
5.6.8	Pathology	Yes
5.6.8.1	Organ Weights	Liver and both kidneys
5.6.9	Histopathology	Urinary bladder only was examined.
5.6.10	Other examinations	For determination of cell proliferation all rats were injected 2 hours prior to necropsy with an intraperitoneal dose of 100 mg/kg 5'-bromo-2'-deoxyuridine (BrdU). For determination of proliferation rate immunohistochemical counting of urothelial cells was compared to sections of the duodenum and uterus.
5.7	**Statistics**	Evaluation of data was performed using SAS software for continuous random variables using the Dunnett test, Adjusted Welch test, or Kruskal-Wallis test followed by adjusted U tests. The statistical test performed for each comparison was not described.
5.8	**Further remarks**	
		RESULTS AND DISCUSSION
5.9	**Body weight**	No effect
5.10	**Food consumption**	No effect
5.11	**Water consumption**	No effect
5.12	**Clinical signs**	Two animals in the treatment group showed increased urine excretion regarded as test substance related; however, mean water consumption was not different.
5.13	**Macroscopic investigations**	No effect
5.14	**Urinalysis**	Calcium imbalance noted in second week, and increased protein excretion at 5 weeks indicated treatment-related effects on the urinary system.

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5.15	**Organ Weights**	Kidney weights were significantly increased after 4 weeks of treatment. Liver weights were significantly increased after 1 and 4 weeks of treatment.
5.16	**Histopathology**	Focal hyperplasia of the transitional epithelium of the urinary bladder was found in one of the 30 rats treated for 4 weeks.
5.17	**Other examinations**	The mean BrdU labelling increased 3.7 fold over controls at 5 weeks in the treated animals; among the 30 treated animals, 5 rats demonstrated labelling more than 2 fold above the maximum value of untreated animals. These animals might be considered potential responders since there was no correlation between BrdU labelling and TFBA metabolite concentration in the urine.
5.18	**Positive control**	When compared to negative controls, NaC (a well known non genotoxic urothelial proliferation inducer) administration produced higher water consumption, increased excreted urinary volume, decreased protein, potassium, and chloride concentration, and lowered total calcium excretion. Cell proliferation of the urinary bladder epithelium was increased 2.4-fold, while 2/10 rats had minimal or slight focal hyperplasia and 3/10 rats had single cell necrosis of transitional cells after 4 weeks of exposure.
5.19	**Other**	No effect on survival was noted.

6 APPLICANT'S SUMMARY AND CONCLUSION

6.3	**Materials and methods**	5000 ppm NAK-4455 (common name transfluthrin) was administered to female rats for 1 week (20 animals) and 4 weeks (30 animals) in the diet. Urinalysis was performed after the dosing interval. The animals were then sacrificed and the urinary tract histologically examined. The test was intended to examine the urinary system in rats after high dose treatment and as such is not covered by a specific guideline. The study uses acceptable numbers of animals for statistical analysis, was well conducted, and has appropriate positive and negative controls.
6.4	**Results and discussion**	Treatment-related effects were seen on the urinary system, including increased urine excretion in some animals, decreased urine calcium concentration and excretion, increased protein excretion, and increased kidney weights at 4 weeks. The treatment had no effect on survival, body weight, and food consumption. There was no dose-response, only a single high dose was used. The study clearly indicates that NAK 4455 induces proliferation in the urinary bladder epithelium, and alters kidney function, in female rats exposed to a high dose for 4 weeks. The positive control NaC a well known product used for non genotoxic induction of urothelial proliferation, produced similar effects.
6.5	**Conclusion**	The study clearly indicates that NAK 4455 induces proliferation in the urinary bladder epithelium in female rats exposed to a high dose for 4 weeks with a NaC toxicity profile which is consistent with a non genotoxic mechanism of urothelial proliferation induction.

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6.5.2	Reliability	1
6.5.3	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7-7-2008
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	
Conclusion	revealed that the mitotic index, as measured by incorporation of bromodeoxyuridine (BrdU), was increased. No correlation was found between tetrafluorobenzoic acid (TFBA) concentrations in urine and the degree of induction of cell proliferation. 12-07-2010 Based on new data the conclusion is adjusted, see discussion in Doc IIA, §3.7
Reliability	
Acceptability	acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table A6.10-1. Results for 4 week study of NAK 4455 in rats

		(-) control	**NAK 4455**	**(+) control, NaC**
Average body weight (g)	0 weeks	218	218	220
	1 week	220	217	222
	4 weeks	229	225	232
Intake	Food/day (g/kg bw)	69/65	65/66	74/65
(1week/4 weeks)	Test compound/day mg/kg bw	0/0	327/328	7.38/6.47
	Water/day (g/kg bw)	89/82	89/85	96/95
Urinalysis	Volume, ml	11.0/7.5	9.5/8.1	8.7/11.0
(2 weeks/5 weeks)	Protein, mg	1.6/1.9	**1.5/2.3***	1.5/2.4
	Na	0.16/0.84	0.09/0.85	0.12/0.96
	K	0.71/2.19	0.66/2.31	0.65/2.4
	Ca	14/49	**8*/41**	**6*/55**
	Cl	0.18/1.14	0.14/1.15	0.18/1.26
Absolute kidney weight (mg)	1 week	1358	1392	1402
	4 weeks4	1413	**1535****	1471
BrdU labelling		n/a	Index increased 3.7-fold over controls	Index increased 2.4-fold over controls
Hyperplasia of urinary bladder epithelium		0 of 30	1 of 30	2 of 10; plus single cell necrosis in 3 of 10

* $p < 0.05$; ** $p < 0.01$

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127 REFERENCE

127.1	**Reference**	[redacted] (2002). Effects of Tetrafluorobenzoic acid (TFBA), NAK 4723) a metabolite of Transfluthrin (NAK 4455), on rat urinary bladder epithelium in vitro. [redacted]. [redacted] Report No. PH 31925 [BES Ref: MO-04-0000050] Report date: April 08, 2002. Unpublished.
127.2	**Data protection**	Yes
127.2.1	Data owner	Bayer AG
127.2.2		
127.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its inclusion on Annex I

128 GUIDELINES AND QUALITY ASSURANCE

2.1	**Guideline study**	No The purpose of the study was to test the cytotoxic potential of TFBA on rat bladder epithelium *in vitro*. Therefore, TFBA was tested in primary rat bladder epithelial explant cultures for its cytotoxic potential. Cytotoxicity was assessed in comparison to the cytotoxic positive control chemical Mitomycine. In parallel, cytotoxicity was measured using a permanent fibroblast cell line of the mouse (3T3). In addition, cytotoxicity measurements in the rat bladder epithelial explant cultures were confirmed by the determination of the DNA content at the end of treatment. There are no applicable guidelines for this purpose. The study was well conducted and controlled and is therefore considered to be an acceptable study.
128.1	**GLP**	No
128.2	**Deviations**	Not applicable

129 MATERIALS AND METHODS

3.1	**Test material**	Tetrafluobenzoic acid (TFBA, NAK 4723), a metabolite of Transfluthrin (NAK 4455)
3.1.1	Lot/Batch number	TFBA was purchased from Aldrich (Cat. No. 303623)
3.1.2	Specification	**Not specified**

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3.1.2.1	Description	**Not specified**
3.1.2.2	Purity	**Not specified**
3.1.2.3	Stability	**Not specified**
3.2	**Study Type**	*In vitro* cytotoxicity test in rat bladder epithelial cells
129.1.1	Organism/cell type	Rat bladder epithelial explant cultures

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129.1.2	Deficiencies / Proficiencies	Not applicable
129.1.3	Preparation of cultures	**Culture of 3T3 cells** 3T3 cells (Clone A31, ATCC Cat. No. CCL-163) were cultivated in DMEM (containing Glutamax, Sodium Pyruvate, Pyridoxine and 4.5g/l Glucose; Gibco Cat. No. 31966-021), supplemented with 10% foetal calf serum (PCS) and Penicillin/Streptomycin. Cells were passaged every 2-3 days by trypsinization and routinely diluted at a rate of 1:3. Cells were cultivated in a humidified incubator at 37°C and 5% CO_2. **Rat bladder epithelial explant cultures** Explant cutures were prepared according to Johnson et al. (1985)[16], with several modifications. Bladders were removed aseptically from neonatal Wistar rats (5-7 days old). Approximately 1mm^2 size pieces were placed into 48 well plates pretreated as follows: plates were coated with collagen IV and fibronectin, dried, and the surface scratched with a sterile scalpel to facilitate adherence of the explants. 400µl of medium was pipetted into each well. The medium used was composed of Ham's F12 containing 0.1% FCS, supplemented with 25U/ml penicillin, 25µg/ml streptomycin, 5µg/ml transferrin, 10µg/ml insulin, 0.1mM non essential amino acids, 1µg/ml hydrocortisone, 10ng/ml mouse EGF and 2mM glutamine. Explant cultures were cultivated in a humidified incubator at 37°C and 5% CO_2 for 5 days before starting the treatment with the test compounds. After 5 days of outgrowth, a viability assay was performed on the living cells using Alamar Blue. After removing the Alamar Blue solution the explants were treated with compound diluted in medium. After 3 days of treatment, another Alamar Blue assay was done and medium changed and replaced again with fresh medium containing test compound for another 3 days. Treatments were done on at least 5 replicate explants per concentration/untreated control. **Collagen IV and fibronectin coating of tissue culture plates** Collagen IV (Sigma Cat.No. 27663) was dissolved at 0.7 mg/ml in 0.05N HCL. Fibronectin (Sigma F-0635) was dissolved at 20µg/ml in distilled water. The solutions were mixed 1:1. 200µl per well were added to 48 well tissue culture plates (Falcon Cat.No. 3078) and incubated (and dried) over night at room temperature. Plates were stored at 4 °C.
129.1.4	Positive control	Mitomycine (purchased from Serva, Cat. No. 29805)

[16] Johnson M. D., Bryan G.T. and Reznikoff C.A. (1985). Serial cultivation of normal rat bladder epithelial cells in vitro. Journal of Urology 133:1076-1081.

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129.2 Administration / Exposure; Application of test substance

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129.2.1 Treatment

Treatment of 3T3 cells

Cells were seeded into 96 well plates at a density of 1x 10^4 cells per well and allowed to attach and grow for 24 hours. Medium was removed and replaced with medium containing test compound at a range of concentrations. Each concentration was applied in quadruplicate wells. Blank wells without cells and solvent control wells containing cells were also included. After 3 days of treatment, an MTT test was performed. The solvent control treated cells were set to 100% viability.

Alamar Blue viability test

The Alamar Blue (AB) assay was performed on living explant cultures before and after 3 and 6 days of treatment. Innate metabolic activity of living cells results in a chemical reduction of AB. Continued growth maintains a reduced environment, while inhibition of growth maintains an oxidized environment. Reduction related to growth causes AB to change from the oxidized (non fluorescent, blue) form to the reduced (fluorescent, red) form.

MTT cytotoxicity test

The MTT test is based on the capability of mitochondrial dehydrogenases of viable cells to cleave the tetrazolium ring of water soluble, yellow MTT (3-[4, 5 dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide), yielding insoluble, purple formazan crystals. The crystals are dissolved in acidified isopropanol and spectrophotometrically measured at 570nm using 630nm as reference wavelength. 20µl of MTT solution (5mg/ml in PBS) was added to the medium of each well of a 96 well plate and incubated at 37 ºC in a humidified incubator containing 5% CO_2 for 3 hrs. Medium was removed and the cells were solubilised by addition of 20µl 3% SDS in water, mixed, and dissolved by addition of 100µl 0.7% HCL in Isopropanol. Plates were shaken on a microtiter plate shaker for 15 min to dissolve blue formazan crystals and optical density was measured.

Determination of DNA content

Determination of DNA content was performed using the FluoReporter Blue Fluorometric dsDNA Quantitation Kit (Molecular Probes, Cat.No. F-2962) according to the manufacturers instructions. Microplate wells containing explant cultures were emptied by overturning onto paper towels and frozen to -80ºC until ready to be measured. Plates were thawed to RT, 100µl distilled water added and plates incubated at 37 ºC for 1h. Plates were again placed at -80 ºC until frozen and thawed to RT to lyse the cells. 100µl of Hoechst 33258 dye solution was added and fluorescence measured at 360nm excitation and 460nm emission wavelength. Blanks (without cells) were subtracted from measured values, and the mean of controls (untreated explants) set to 100%.

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129.2.2 Way of application	Solutions of TFBA in dimethylsulphoxide (DMSO) at a range of concentrations up to a maximum of 1000µg/ml and Mitomycine in water at a concentration of 100µg/ml were applied directly to cells within the culture medium.

130 RESULTS AND DISCUSSION

4.1 Cytotoxicity

Cytotoxicity of 3T3 cells

TFBA was only slightly cytotoxic at the highest test concentrations, with a no effect concentration of 300µg/ml and an IC50 of >1000µg/ml

The solvent DMSO was only marginally cytotoxic at the highest concentration used (75% viability at 1% DMSO). At higher concentrations DMSO was cytotoxic to 3T3 cells with an IC50 of 2%.

Cytotoxicity of epithelial explant cultures

Rat bladder epithelial explant cultures were not sensitive to TFBA treatment up to a concentration of 100µg/ml. At 300µg/ml viability was reduced to 50%. Explant cultures were found to be dead after 3 days of treatment with 1000µg/ml TFBA. As a cytotoxic control compound, Mitomycine was used at 100µg/ml, which reduced viability to zero after 3 days of treatment. In contrast, DMSO had no cytotoxic effect on the explant cultures, even if applied at the concentration of 10%, which was strongly cytotoxic to 3T3 cells. No significant difference was observed between results of 3 days or 6 days of treatment.

DNA content of explant cultures

DNA content was determined after 3 or 6 days of treatment, following the last AB assay. DNA content was not affected up to 300µg/ml TFBA. At 1000µg/ml however, the DNA was reduced to background levels. In addition, treatment with Mitomycine for 3 days decreased the DNA content to zero due to cytotoxicity, whereas 10% DMSO had no effect compared to untreated controls.

Discussion

Rat bladder epithelial explant cultures were sensitive to TFBA at a concentration of 300µg/ml, with a no effect concentration of 100µg/ml. This effect was not related to the acidic character of TFBA, since addition of high concentrations of TFBA to medium did not reduce its pH (pH 6.5-7). The explant cultures are much more sensitive to TFBA than cells of the mouse fibroblast cell line 3T3, that are used as a well established reference cell line. These cells were insensitive up to 300µg/ml and showed an IC50 of more than 1000µg/ml.

The data obtained using the Alamar Blue cytotoxicity test were confirmed by measurement of DNA content at the end of the treatment/culture period. The DNA content was unaffected up to 300µg/ml TFBA. At 1000µg/ml DNA was reduced to background levels.

Results from 3T3 cytotoxicity tests were reproduced in at least 2 independent experiments.

Data on the rat bladder epithelial explant cultures (including AB cytotoxicity and DNA content) were reproduced in 4 independent experiments.

131 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods

The test material is tetrafluorobenzoic acid (TFBA), which is a metabolite of the pyrethroid Transfluthrin (NAK 4455).

The test material was dissolved in DMSO. TFBA at a range of concentrations up to a maximum concentration of 1000µg/ml was tested in primary rat bladder epithelial explant cultures for its cytotoxic potential. In parallel, cytotoxicity was measured using a permanent fibroblast cell line of the mouse (3T3). In addition, cytotoxicity measurements in the rat bladder epithelial explant cultures were confirmed by the determination of the DNA content at the end of treatment.

The negative (solvent) and positive controls were run concurrently. The positive control substance, Mitomycine at 100µg/ml was used to confirm the sensitivity of the test system.

131.1 Results and discussion

TFBA was not cytotoxic up to a concentration of 100µg/ml, using the Alamar Blue viability assay. The growth of primary explant cultures of rat bladder epithelial cells was inhibited at 300µg/ml, and explants were dead at 1000µg/ml. The results closely resemble the results of the determination of the DNA content on the last day of the culture, which was slightly less sensitive with a no effect concentration of 300µg/ml. At the concentration of 1000µg/ml TFBA, DNA content was reduced to background levels. The results of the highest test concentration of TFBA were identical to the results of the positive cytotoxic control, Mitomycine, at 100µg/ml. The cytotoxic effects on explant cultures are stronger compared to cytotoxicity on the 3T3 mouse fibroblast cell line, which showed a no effect concentration of 300µg/ml and an IC50 concentration of more than 1000µg/ml.

131.2 Conclusion

The results of this study supports the hypothesis that high concentrations of TFBA, the main metabolite of Transfluthrin, may lead to irritation of the urinary bladder epithelium, which in the long term may result in hyperplasia and tumours *in vivo*. In fact, the mean urine TFBA concentrations calculated in high dose treated rats in the carcinogenicity study showed that urinary TFBA concentration lay within a range of 500µg/ml up to more than 1500µg/ml depending on the age of the rats and the urinary volume. This concentration is clearly cytotoxic *in vitro* and may thus explain the effects observed *in vivo*

131.2.1 Reliability **2**

131.2.2 Deficiencies No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	7-7-2008
Materials and Methods	The version of the applicant is acceptable.

Results and discussion	TFBA was not cytotoxic up to 0.1 mg/ml. Growth of explants was inhibited as from 0.3 mg/ml TFBA. For comparison, the TFBA concentration in urine of rats dosed 327 mg/kg bw/d for 4 weeks (proliferation study , was about 0.5 mg/ml, which is 5 times higher than the no-effect concentration in the in vitro test.
Conclusion	12-07-2010 Based on new data the conclusion is adjusted, see discussion in Doc IIA, §3.7
Reliability	
Acceptability	acceptable
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

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	132 REFERENCE	
132.1	**Reference**	[redacted] (2010) A subchronic study in rats and mice to investigate the mechanism of bladder tumors with technical grade transfluthrin, [redacted], Study report No: 09-S72-RQ, Report date February 25, 2010 (unpublished). **BES Reference: Not yet allocated**
132.2	**Data protection**	Yes.
132.2.1	Data owner	Bayer CropScience AG
132.2.2	Companies with letter of access	None
132.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
	133 GUIDELINES AND QUALITY ASSURANCE	
133.1	**Guideline study**	No, mechanistic study
133.2	**GLP**	Yes
133.3	**Deviations**	None
	134 MATERIALS AND METHODS	
134.1	**Test material**	Transfluthrin
134.1.1	Lot/Batch number	ABIDTBN019
134.1.2	Specification	As given in section 2
134.1.2.1	Description	White melt
134.1.2.2	Purity	99.6% w/w
134.1.2.3	Stability	As given in section 2
134.2	**Test Animals**	
134.2.1	Species	Rat and Mouse
134.2.2	Strain	Wistar Hanover IGS [CRL: WI (Han)] rats (nulliparous and nonpregnant) B6C3F1 mice rats (nulliparous and nonpregnant)
134.2.3	Source	[redacted]
134.2.4	Sex	Female
134.2.5	Age/weight at study initiation	Rats 8 weeks/ 150-193 g (start of treatment) Mice 8 weeks/ 18-22 g (start of treatment)
134.2.6	Number of animals per group	10 animals/group

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134.2.7	Control animals	Yes
134.3	**Administration/ Exposure**	Oral (diet)
134.3.1	Duration of treatment	4 weeks for rat groups 1, 3, 5, 6, 7 and both mouse groups, 13 weeks for rat groups 2, 4.
134.3.2	Frequency of exposure	Continuous (diet, *ad-lib*)
134.3.3	Post exposure period	None
134.3.4	Type	Dietary
134.3.5	Concentration	Rats: 0, 2000, 5000, 0, 5000 ppm over 4 weeks for groups 1, 3, 5, 6 ,7 equating to 0, 180, 454, 0 and 542 mg/kg bw, respectively 0, 2000 ppm over 13 weeks for groups 2 , 4 equating to 0, 435 mg/kg bw, respectively Mice: 0, 1000 ppm over 4 weeks, equating to 0, 401 mg/kg bw, respectively
134.3.6	Vehicle	Acetone was used to add the test compound to the diet
134.3.7	Concentration in vehicle	N/A
134.3.8	Total volume applied	N/A
134.3.9	Controls	vehicle, plain Altromin 1321diet or Altromin 1321 diet + 1.25% NH_4Cl
134.4	**Examinations**	
134.4.1	Observations	
134.4.1.1	Clinical signs	Yes, observed twice daily (except weekends and holidays when animals were observed once a day). A detailed clinical examination was performed once pre-treatment and weekly thereafter
134.4.1.2	Mortality	Yes, observed twice daily (except weekends and holidays when animals were observed once a day)
134.4.2	Body weight	Yes, weekly, and prior to necropsy
134.4.3	Food consumption	Yes, calculated weekly
134.4.4	Water consumption	No
134.4.5	Ophthalmoscopic examination	No
134.4.6	Haematology	No
134.4.7	Clinical Chemistry	No
134.4.8	Urinalysis	Yes, over a 24 hour period during study week 3 for mice and during study week 4 for rats scheduled for sacrifice after 4 weeks of treatment. Parameters: pH, creatinine and tetrafluorobenzoic acid (TFBA) During study week 3, freshly voided urine was collected from all rats scheduled for sacrifice after 4 weeks of treatment.

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Parameters: pH and microcrystal analysis

134.5 Sacrifice and pathology

134.5.1	Organ Weights	Yes at scheduled sacrifice, urinary bladder (after fixation), section of small intestine (after fixation), kidneys and liver were weighed for all animals at scheduled sacrifice
134.5.2	Gross and histopathology	One hour prior to scheduled sacrifice after 4 and 13 weeks of treatment, all animals were injected IP with BrdU in 0.9% saline. At necropsy, the urinary bladder, section of small intestine, kidney and liver were examined macroscopically. The urinary bladder and section of small intestine were preserved in Bouin's fixative. The kidney and liver were preserved in 10% buffered formalin. One half of the bladder was processed for scanning electron microscopy. The other half of the urinary bladder together with the intestinal tissue, kidney and liver were processed for histopathological examination. Approximately 4-5 micron sections of tissue were stained with hematoxylin and eosin and examined histopathologically. Unstained slides of urinary bladder/intestinal tissue were retained for determination of bromodeoxyuridine labelling index assessment. The intestinal tissue served as a positive control for this procedure.
134.5.3	Statistics	Body weight (in-life), food consumption, and pH were analyzed by Bartlett's test for homogeneity. If the data were homogeneous, an ANOVA was performed by Dunnett's t-test on parameters showing a significant effect by ANOVA. If the data were non-homogeneous, a Kruskal-Wallis ANOVA was performed, followed by the Mann-Whitney U-test to identify statistical significance between groups. Statistical significance was determined at $p \leq 0.05$ for all tests with the exception of Bartlett's test, in which a probability value of $p \leq 0.001$ was used. All tests were two-tailed. Group means for terminal body weights, urinary pH, creatinine concentrations, organ tissue weights, and labeling indices were evaluated using ANOVA, followed by Duncan's multiple range test for group-wise comparisons. Histopathology was compared using the 2-tailed, Fisher's Exact test. SEM data were analyzed using 1-way nonparametric procedures followed by a Chi Square test. P values less than 0.05 were considered significant.

135 RESULTS AND DISCUSSION

135.1	**Observations**	
135.1.1	Clinical signs	No effects
135.1.2	Mortality	No deaths
135.2	**Body weight gain**	No effects
135.3	**Food consumption and compound intake**	No effects
135.4	**Blood analysis**	

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135.4.1 Haematology — Not assessed

135.4.2 Clinical chemistry — Not assessed

135.4.3 Urinalysis

pH

Altromin 1321 produced markedly alkaline urine in rats, which is consistent with the previous findings, with urinary pH in all of these groups (freshly-voided and 24-hour collection) of 8.5 or 8.6. For freshly-voided urine, the urinary pH in the control group fed Altromin 1321 supplemented with NH_4Cl was 8.2, compared with a substantial decrease (pH = 6.7) in the group fed Altromin 1321 supplemented with NH_4Cl and 5000 ppm of transfluthrin. For the urine collected over a 24-hour period, the pH for the Altromin 1321 + NH_4Cl control group and the 5000 ppm transfluthrin + NH_4Cl group was significantly reduced (pH = 7.5 and 7.3 for the control and treated groups, respectively).

Creatinine

Treatment with 5000 ppm of transfluthrin in the diet was associated with an increase in urinary creatinine, with urinary concentrations of 62% (standard Altromin diet) or 52% (acidified diet) above controls. However, these differences from the respective control groups were not statistically significant and thus were considered not to be compound-related. Treatment with transfluthrin at 2000 ppm in rats or 1000 ppm in mice had no effect on the urinary creatinine concentration.

Micro Crystals (Urinary Filters)

$MgNH_4PO_4$ crystals, which are normally present in the urine of rats, were not found in the urine of any transfluthrin-treated or untreated rats in this study.

Calcium-containing crystals, mainly with ball or dumbbell shaped morphologies, were present in the urine of rats from all (control and treated) groups. The number of filters with calcium-containing crystals was decreased in the group treated with 5000 ppm transfluthrin + NH_4Cl, compared to the control group that received acidified diet and compared to the group treated with 5000 ppm transfluthrin in Altromin 1321 without NH_4Cl. The number of filters with calcium oxalate crystals was higher in the 5000 ppm transfluthrin group, compared to the control group. Addition of NH_4Cl to the diet decreased the number of filters with calcium oxalate crystals in the transfluthrin group, as compared to the transfluthrin group without NH_4Cl. There were no calculi found on any filters.

Tetrafluobenzoic Acid (TFBA)

The concentration of TFBA in rat urine for the 2000 ppm and 5000 ppm (with or without NH_4Cl) dose groups were approximately 2- and 4-times higher, respectively, than the concentration of TFBA in the urine of mice that received 1000 ppm of transfluthrin.

135.5 Sacrifice and pathology

135.5.1 Organ weights — Rats

The absolute and relative bladder weights in the 5000 ppm transfluthrin-

treated group were slightly greater than control, but as the differences were small and not statistically significant and the slightly greater bladder weight was not considered to be compound-related. The absolute and relative bladder weights in the 5000 ppm transfluthrin + NH_4Cl treated group were slightly decreased compared to the 0 ppm transfluthrin + NH_4Cl group, but neither difference was statistically significant and were considered not to be compound-related.

Treatment with transfluthrin (with or without NH_4Cl) caused an increase in the relative kidney weight compared to the respective control groups. This difference from control was statistically significant only in the group treated with transfluthrin without NH_4Cl. As there was no concurrent statistical or biologically significant increase in absolute weight, there was no compound-related effect on kidney weight.

The absolute and relative liver weights were statistically significantly increased in the groups treated with 2000 ppm transfluthrin or 5000 ppm transfluthrin with or without NH_4Cl compared to the respective control group and were considered to be compound-related.

Mice

Organ weights for mice treated with 1000 ppm transfluthrin for 4 weeks were similar to the control group, except for a statistically-significant increase in the absolute and relative liver weight that was considered to be compound-related. After treatment with 2000 ppm transfluthrin for 13 weeks, there was no effect on the absolute and relative bladder or kidney weights, but there was a statistically significant increase in the absolute and relative liver weights that was considered to be compound-related.

135.5.2 Gross and histopathology

Gross Pathology

The were no remarkable gross pathology findings

Histopathology

Urinary Bladder

After treatment for 4 weeks, there was no increase in the incidence of simple hyperplasia in the urinary bladder or in the BrdU labeling index in any rats or mice in any transfluthrin treatment group, as compared to the concurrent control. There were no test substance-related alterations in the SEM classification of the bladders in rats treated with 2000 or 5000 ppm transfluthrin. There was an increase in the number of class 5 bladders by SEM in the group treated with 5000 ppm transfluthrin + NH_4Cl compared to the NH_4Cl control group or the group treated with 5000 ppm transfluthrin in the non-acidified diet. However, the increase did not result in a statistically-significant difference in the SEM classification for the treated group (Table A6.10/03-1). Due to the high number of class 5 bladders (7/10) in the mouse control group, it was not possible to evaluate the effects of transfluthrin on the mouse bladder by SEM (Table A6.10/03-2).

After treatment for 13 weeks, there was no increase in the incidence of simple hyperplasia or in the BrdU labeling index. However, examination by SEM showed an increase in superficial cytotoxicity and necrosis of the bladder of the treated rats (2000 ppm) compared to the control group and an increase in the number of bladders with an accumulation (piling up) of

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round cells indicative of hyperplasia. As a result of these changes, the SEM classification for the transfluthrin-treated group was significantly different compared to the control group (Table A6.4.10/01)

Kidney and Liver

There were no remarkable histopathology findings in the kidney or liver for rats or mice (Table A6.4.10/02).

136 APPLICANT'S SUMMARY AND CONCLUSION

136.1 Materials and methods

Groups of 10 female Wistar rats were dosed with transfluthrin at 2000 ppm or 5000 ppm for 4 weeks or at 2000 ppm for 13 weeks. A further group of 10 female rats received 5000 ppm transfluthrin in diet containing 1.25% ammonium chloride for 4 weeks, to assess the effect acidifying the alkaline diet and thereby lowering urinary pH, would have on microcrystal formation the toxicity of the primary metabolite of transfluthrin; TFBA. Transfluthrin was also administered to a group of 10 female B6C3F mice at 1000 ppm of transfluthrin for 4 weeks. Corresponding control controls received the relevant transfluthrin-free diet.

During the study, body weights and food consumption were measured weekly. Animals were examined daily for moribundity and mortality and were examined weekly for detailed clinical observations. During study weeks 3 and 4, urine was collected from mice and rats, respectively, in metabolism cages for a 24-hour period and analyzed for TFBA, pH, and creatinine. During week 3, freshly-voided urine was collected from the rats, the pH was determined and the urine was analyzed for the presence of microcrystals. During study Weeks 5 (rats and mice) and 14 (rats), bromodeoxyuridine (BrdU) was administered by intraperitoneal injection (IP) 60 minutes prior to euthanasia. At necropsy, the liver, kidneys, urinary bladder and small section of duodenum were weighed; these tissues were collected for histological evaluation. Cell proliferation of the urinary bladder and small intestine was evaluated. The urinary bladder was also evaluated by scanning electron microscopy (SEM).

136.2 Results and discussion

There were no compound-related clinical signs, no animals died or were sacrificed *in-extremis* during the study, and there were no compound-related effects on body weight or food consumption for rats or mice.

Rats: Urinary pH was extremely high, with a mean pH of approximately 8.5 in the control and treated groups receiving standard Altromin diet. In the control group fed acidified Altromin diet, the mean pH was 7.5 and 8.2, whilst in the 5000 ppm of transfluthrin group fed acidified Altromin diet the mean pH was 6.7 and 7.3. The high pH was due to the use of Altromin 1321 diet.

There was no compound-related effect on urinary creatinine concentration.

Evaluation of urinary solids showed no $MgNH_4PO_4$ crystals present in the urine of treated or untreated rats. However, dumbbell and ball-shaped calcium-containing crystals were present in all groups with similar frequency and similar amounts of crystals per filter. Calcium oxalate crystals were also present in all transfluthrin-treated groups, and treatment with 5000 ppm of transfluthrin increased the incidence of the calcium oxalate crystals. The presence of calcium-containing crystals in the urine of

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all groups, including controls, was likely due to the high urinary pH associated with the use of the Altromin diet. Since the crystals were similar in all groups, it is unlikely that they are the cause of the cytotoxicity observed with 2000 ppm of transfluthrin treatment at 13 weeks. The presence of calcium-containing crystals in the control and treated group where NH_4Cl was added to the diet is consistent with the finding that the acidified diet did not consistently lower the urinary pH of individual animals in these groups to below 6.5, a critical level for the formation of crystals.

At the 5-week interim sacrifice, following 4 weeks of treatment, bladder and kidney weights were unaffected by treatment with transfluthrin. A compound-related increase in liver weight was observed in all 3 transfluthrin treated groups. No increase in simple hyperplasia in the urinary bladder epithelium or on BrdU labeling index was observed in any of the 3 transfluthrin treated groups. However, examination of the bladder surface by SEM did show a non-statistical, but compound-related, increase in the number of bladders with an accumulation (piling up) of immature, round cells, which is indicative of hyperplasia, in the 5000 ppm transfluthrin + NH_4Cl group. No changes were seen at the SEM examination in the remaining two treated groups.

At the 14-week sacrifice, following 13 weeks of treatment with 2000 ppm of transfluthrin in standard Altromin diet, there was no effect on bladder or kidney weight, but there was a statistically significant increase in absolute and relative liver weight. No effect on bladder epithelium was observed when examined by light microscopy or when examined using BrdU labeling. However, examination by SEM showed an increase in cytotoxicity, as evidenced by necrosis and exfoliation of the superficial cells. The effect was statistically significant when graded using the classification system specified.

Thus, no effects were seen on the bladder epithelium after 4 weeks of treatment with either 2000 ppm or 5000 ppm of transfluthrin in standard diet. However, lowering urinary pH with the concomitant administration of NH_4Cl in the diet did appear to lead to some effect with the administration of 5000 ppm of transfluthrin, as detected by SEM. By 13 weeks, there was clear evidence of cytotoxicity when 2000 ppm of transfluthrin was administered in standard Altromin diet. This effect occurred at a dose level at which bladder tumors were detected in the 2-year bioassay. Given the weak tumorigenic effect toward the rat bladder, the mild effect on the urothelium through 13 weeks of treatment is what would be expected, as has been shown for non-genotoxic bladder carcinogens in the rat.

Detection of superficial necrosis by SEM without detection by light microscopy is common. The superficial layer is very thin and difficult to assess by light microscopy. Furthermore, preparation of the urinary bladder for light microscopic examination frequently leads to focal tearing and loss of the superficial layer. By light microscopy, this may not be detectable or may not be distinguishable from cytotoxic effects. SEM examination avoids these problems, and has the added benefit of providing vastly more of the bladder for examination. Using this procedure enables the surface of an entire half of the bladder to be examined, in contrast to the thin strips (5-6 µm sections) available when the bladder is examined by light microscopy.

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Thus, SEM examination is both more sensitive and specific for the evaluation of the urothelium for superficial cytotoxicity.

In summary, 13 weeks of treatment with transfluthrin in the diet at a dose of 2000 ppm to female Wistar rats induces superficial cytotoxicity. The effect of urinary acidification is unclear, but it may have an enhancing effect. This would be more supportive of a chemical toxic effect than an effect due to formation of urinary calcium-containing crystals.

Mice: Treatment of mice with transfluthrin had no effect on the urinary creatinine level, bladder or kidney weights, incidence of urothelial hyperplasia, or the BrdU labeling index. There was a compound-related increase in liver weights in the transfluthrin-treated group. It was not possible to interpret the SEM examination of the bladder surface of the transfluthrin-treated mice due to extensive changes in the bladder surface of the control mice. This was most likely due to the high urinary pH (> 8.0 in both the control and treated group) associated with the Altromin diet and possibly due to the handling of the mice, including single housing.

TFBA: TFBA concentrations in rat urine compared to mouse urine were approximately 2 times higher for rats administered 2000 ppm (180 mg/kg/day) of transfluthrin and were approximately 4 times higher for rats administered 5000 ppm of transfluthrin (with or without NH_4Cl; 454 and 542 mg/kg/day, respectively) as compared to mice that were administered 1000 ppm (401 mg/kg/day) of transfluthrin. The lower concentration of TFBA in the urine of mice dosed with 1000 ppm of transfluthrin may explain or at least contribute toward the difference in the occurrence of urinary bladder tumors observed in the chronic rat and mouse studies.

136.3 Conclusion

It appears that the mechanism of action for the induction of tumors in the urinary bladder of rats is due to high concentrations of the major metabolite of transfluthrin (i.e., TFBA) in the urine.

136.3.1 LO(A)EL	Not relevant
136.3.2 NO(A)EL	Not relevant
136.3.3 Reliability	1
136.3.4 Deficiencies	No

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	EVALUATION BY COMPETENT AUTHORITIES
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2010
Materials and Methods	The version of the applicant is acceptable
Results and discussion	
Conclusion	RMS supports the conclusion.
Reliability	
Acceptability	Acceptable
Remarks	See doc IIIA 6.10 appendices with position papers mechanistic considerations dated 19-02-2010 and 10-05-2010. Furthermore the appendix interpretation of short-term assays regarding the effects of transfluthrin on rat urethelium in vivo and in vitro by [redacted] March 12, 2010. RMS supports the conclusions described in the position papers.
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.10/03-1: Effects on the bladder of female Wistar rats after treatment with transfluthrin

Treatment	Histopathology			SEM Classification				
	Normal	Simple Hyperplasia	Labeling Index (%)[a]	1	2	3	4	5
Week 5								
0 ppm transfluthrin	10	-	0.07±0.01 (8)	3	5	2	-	-
2000 ppm transfluthrin	10	-	0.08±0.02 (8)	3	6[b]	1	-	-
5000 ppm transfluthrin	9	1	0.07±0.01 (9)	2	4	3	1	-
0 ppm transfluthrin + 1.25% NH_4Cl	7[c]	3	0.03±0.01 (9)[d]	4	3	3		
5000 ppm transfluthrin + 1.25 % NH_4Cl	10	-	0.05±0.02 (7)	2	3	-	1	4[e]
Week 14								
0 ppm transfluthrin	10	-	0.04±0.02 (8)	4	4	2	-	-
2000 ppm transfluthrin [f]	9	1	0.04±0.02 (9)	2	1	5	1	1

[a] Results expressed as the mean ± S.E. (n)

[b] Calcium and phosphorus-containing crystal (30 µm diameter) present on the surface of the bladder.

[c] Focal submucosal chronic inflammation present in one bladder.

[d] Significantly different from 5 week 0 ppm transfluthrin group, $p<0.05$.

[e] Occasional apoptotic cells present.

[f] SEM classification significantly different from Week 14 0 ppm transfluthrin group, $p<0.05$.

Table A6.10/03-2: Effects on the bladder of female B6C3F1 mice after treatment with transfluthrin

Treatment	Histopathology			SEM Classification				
	Normal	Simple Hyperplasia	Labeling Index (%)[a]	1	2	3	4	5
0 ppm transfluthrin	9	1	0.02±0.01 (10)	3	-	-	-	7
1000 ppm transfluthrin	9	1	0.04±0.02 (10)	-	-	-	1	9

[a] Results expressed as the mean ± S.E. (n)

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Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human

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137 REFERENCE

137.1 Reference	[redacted] (2009) Transfluthrin comparison of the *in vitro* metabolism in liverbeads™ from male rat, mouse, dog and human. [redacted], Study report No: **SA 09122**, Report date 2009-10-23 (unpublished). **BES Reference: M-359222-01-1**
137.2 Data protection	Yes
137.2.1 Data owner	Bayer CropScience AG
137.2.2 Companies with letter of access	None
137.2.3 Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

138 GUIDELINES AND QUALITY ASSURANCE

138.1 Guideline study	No, no guidelines available
138.2 GLP	Yes
138.3 Deviations	No, there were no protocol deviations during the study

139 MATERIALS AND METHODS

139.1 Test material	Transfluthrin [methylene-^{14}C]-Transfluthrin
139.1.1 Lot/Batch number	Transfluthrin: Batch No. ABIDTBN019; Certificate No. AZ 15885 [methylene-^{14}C]-Transfluthrin: Reference synthesis No. SEL/1520, Sample ID No. KATH 6745
139.1.2 Specification	As given in section 2
139.1.2.1 Description	Transfluthrin: white melt [methylene-^{14}C]-Transfluthrin: solid
139.1.2.2 Purity	Transfluthrin: 99.6% [methylene-^{14}C]-Transfluthrin: >99% (radio purity, HPLC), specific activity: 3.67 MBq/mg

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139.1.2.3	Stability	The radiolabeled test substance was stored in air-tight, light-resistant container(s) at approximately -20°C. The non-radiolabeled test substance was stored in air-tight, light-resistant container(s) at room temperature. Under these conditions transfluthrin is stable over 2 years.
139.2	**Preliminary test**	**cytotoxicity evaluation**
139.2.1	Organism/cell type	freshly isolated rat hepatocytes
139.2.2	Preparation	A young male adult Wistar rat was used for hepatocyte isolation. The animal was anesthetized with pentobarbital and an in situ perfusion of the liver was performed using a solution of liberase at 28 µg/ml. The tissue was then mechanically dissociated and the cells collected after filtration on gauze. The cells were washed with HBSS (Hanks Balanced Salt Solution), centrifuged and then resuspended in Hepatozym™ (Gibco) + Penicillin 50 UI/ml and streptomycin 50 µg/ml. Viability was determined using Trypan blue. Cell culture with viability over 75% was used for the experiment. The cells were then plated in 12-well plates coated with collagen at the density of approximately 0.5 million cells per ml. Approximately 1 ml was distributed in each well. The cell cultures were maintained at 37°C under 5% CO_2
139.2.3	Controls	Positive (SDS) and negative (DMSO) controls were also assayed in this experiment.
139.2.4	Concentrations	In the preliminary test, 3 concentrations of Transfluthrin were tested: 25, 100 and 400 µM (two wells per concentration were used).
139.2.5	Evaluation	After approximately 24-hour exposure, the cytotoxicity was assessed using a colorimetric assay based on the reduction of a tetrazolium salt XTT to a formazan product by mitochondrial enzymes
139.3	**Main study**	***in vitro* metabolism**
139.3.1	Organism/cell type	Liverbeads ™ (from rat, mouse, dog and human) Liverbeads™ are immobilized hepatocytes entrapped within an alginate matrix For the rat and dog, one batch of Liverbeads™ was used. For the mouse, two batches (each being a pool of three different cell preparations) were pooled. For human, four batches corresponding to four different donors were pooled before being used in incubations
139.3.2	Source	Biopredic International, Rennes, France
139.4	**Administration / Exposure; Application of test substance**	Non-entry field
139.4.1	Concentrations	25 µM and 250 µM were used The selection of dose concentrations for the *in vitro* metabolism part was based on results obtained in the preliminary cytotoxicity assays in rat hepatocytes
139.4.2	Way of application	For each species:

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The vials containing Liverbeads™ were thawed according to the procedures described by the supplier. Then, the cells were pooled in the medium "1" containing Hepatozym™ (Gibco), insulin (4 µg/ml), glutamine (2 mM), Penicillin 50 UI/ml and streptomycin 50 µg/ml. Fetal Calf Serum (FCS, 10%) was added to this medium.

The Liverbeads™ were seeded in 12-well plates following the design below, and the plates placed in a humidified incubator (37°C, 5% CO_2) for at least 3 hours. At the end of this time period, the medium was removed and replaced by another medium which corresponds to medium "1" + hydrocortisone hemisuccinate 10^{-6}M (and without FCS).

139.4.3 Incubation

The enzymatic reactions were initiated by the addition of the radiolabeled Transfluthrin (alone or mixed with non radiolabeled Transfluthrin) in acetonitrile to achieve the two concentrations, 25 or 250 µM. Then the plates were placed in an incubator at 37°C under gentle shaking.

The time periods for the incubations were 4 and 24 hours. Control incubations without radiolabeled compound were also performed for each species.

139.4.4 Evaluation

At the end of the incubation time, the Liverbeads™ were first dissolved using EDTA Na_2 (100 mM), then the hepatocytes were sonicated for a period of 20 seconds. The hepatocytes plus incubation medium plus dissolved Liverbeads™ from each well were transferred into separate vials and immediately stored at about -20°C until analysis.

139.4.5 Analytical procedures

Vials containing incubations mixtures were centrifuged and the supernatants were directly injected into the chromatographic system to be analyzed using radio-HPLC, mass spectrometry or UV comparison with authentic reference standards.

139.4.6 Metabolite identification

The chromatograms corresponding to the different species and different concentrations were subjected to semi quantification: the peaks corresponding to metabolites were integrated using the software Empower (version 2 build 2154). The area from each peak detected in each species at each incubation time period was calculated (for all duplicates). From these areas, the relative distribution (expressed as percentage) of the detected metabolites in the different samples was calculated with Empower

The identified metabolites were characterized using authentic certified reference compounds (See Table A6.10/04-1)

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Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human

140 RESULTS AND DISCUSSION

140.1 Low concentration experiment (25 µM)

The mean relative distribution of the detected metabolites, expressed as relative percentage of the sum of the areas from the different peaks (corresponding to metabolites/parent) is presented in Table A6.10/04-2

The metabolites detected were TFB acid (TFBA), TFB alcohol and glucuronide-TFB alcohol. The parent compound [methylene-^{14}C]-Transfluthrin was detected in the human Liverbeads™, only at a very low level (0.30%), at the 4-hour incubation time period.

After 4 hours of incubation, Transfluthrin was metabolized to the major metabolite TFB alcohol which accounted for 98.9% in human, 67.7% in the rat, 65.9% in the dog and for 54.5% in the mouse. In a second step, the TFB alcohol was mainly metabolized to glucuronide -TFB alcohol which represented 31.4% of the detected metabolites in the rat Liverbeads™, 34.1% in the dog and 42.4% in the mouse. This glucuronide represented only 0.8% of the detected metabolites in human after 4 hours of incubation. TFBA was only a minor metabolite in the mouse (3.1%) and in the rat (0.9%), and was not detected in the two other species.

After 24 hours of incubation, high amounts of glucuronide -TFB alcohol were measured in the rat (92.1%), in the mouse (93.5%) and in the dog (76.6%). By contrast, in human the level of the glucuronide -TFB alcohol represented only 5.7% of detected metabolites, the major metabolite still being TFB alcohol (94.4%) as previously detected at the 4 hour incubation time point. Compared to the values measured after a 4-hour incubation time period, TFBA was slightly increased in the mouse (5.3%), unchanged in the rat (0.8%) and still not detected in human or dog.

140.2 High concentration experiment (250 µM)

The mean relative distribution of the detected metabolites, expressed as relative percentage of the sum of the areas from the different peaks (corresponding to metabolites/parent) is presented in Table A6.10/04-3

At the high concentration of 250 µM, no new metabolite was detected in any species at either incubation time period, when compared to the low concentration results. Transfluthrin was present in human incubates at high amounts (45.2%) after the 4-hour incubation period. The parent compound was also detected at lower levels in the two other species compared to the 25 µM concentration: i.e., 5.3% in the mouse and 4.2% in the rat.

After 4 hours of incubation, the levels of the major metabolite TFB alcohol in the rat, dog and mouse were higher than those measured at the low concentration of 25 µM for the same incubation period and represented 88.3%, 94.8% and 88.9%, respectively, of the detected metabolites. In addition, the levels of glucuronide-TFB alcohol in the same species were lower than previously observed: 7.3% for the rat, 5.1% for the dog and 4.5% for the mouse, which suggested a saturation of the biotransformation enzymatic system. No glucuronide of TFB alcohol was detected in the human incubates after the 4 hours time period.

As seen previously, TFBA was present at very low levels in two species only, rat and mouse.

After the 24-hour incubation period, the metabolizing processes continued

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and therefore, the parent compound was no longer detected in any species. The major metabolite was TFB alcohol only for human (99.1%); TFB alcohol and its glucurono-conjugated were present in the rat (60.8% and 37.9%, respectively), the dog (86.2% and 13.8%) and the mouse (74.6% and 21.0%).

The level of TFBA was slightly increased compared to the 4 hours incubation time and was still present in the same species: the rat (1.4%) and the mouse (4.5%).

141 APPLICANT'S SUMMARY AND CONCLUSION

141.1 Materials and methods

The comparative *in vitro* metabolic profile of Transfluthrin between different species was assessed using hepatocytes (Liverbeads™) from male rat, mouse, dog and human.

Liverbeads™ were incubated for 4 & 24 hours with [methylene-^{14}C]-Transfluthrin at the concentrations of approximately 25 µM and 250 µM. these doses were selected based on results of a preliminary cytotoxicity test in isolated rat hepatocytes.

Each detected metabolite was analyzed using liquid chromatography/mass spectrometry (LC/MS) analysis where possible and identified with reference standards.

141.2 Results and discussion

The mean relative distribution of the detected metabolites, expressed as relative percentage of the sum of the areas from the different peaks (corresponding to metabolites/parent) is presented in Table A6.10/04-2 and Table A6.10/04-3 At both concentrations tested, 25 µM and 250 µM, the following metabolites were detected in the rat, mouse, dog and human Liverbeads™: tetrafluoro-benzoic acid (TFBA), tetrafluoro-benzylalcohol (TFB alcohol) and tetrafluoro-benzylglucuronide (glucuronide-TFB alcohol).

141.3 Conclusion

The major metabolite was TFB alcohol, being further metabolized into the glucurono-conjugated TFB alcohol. A minor metabolite was TFBA, which was detected in the rat and mouse only. The parent compound was detected occasionally, mainly in human after 4 hours of incubation.

At the low concentration 25 µM

After 4 hours of incubation, Transfluthrin was essentially metabolized in all species with only a very low amount (0.3%) being still present in human Liverbeads™. The major metabolite was found to be tetrafluoro-benzylalcohol (TFB alcohol) which represented 98.9% of the detected metabolites (including also parent) in human, 67.7% in the rat, 65.9% in the dog and 54.5% in the mouse. In a second step, the TFB alcohol was mainly metabolized to glucuronide-TFB alcohol, which represented 31.4% of the detected metabolites in the rat, 34.1% in the dog and 42.4% in the mouse. This glucuronide was only 0.8% of the detected metabolites in human. tetrafluoro-benzoic acid (TFBA) represented a minor metabolite in the mouse (3.1%) and in the rat (0.9%), and was not detected in the other two species.

After 24 hours of incubation, high amounts of glucuronide-TFB alcohol were measured in the rat (92.1%), in the mouse (93.5%) and in the dog

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(76.6%). By contrast, in human the level of the glucuronide-TFB alcohol represented only 5.7% of detected metabolites, the major metabolite still being TFB alcohol (94.4%), as previously detected after 4 hours of incubation. Compared to the values measured after a 4-hour incubation time, TFBA was slightly increased in the mouse (5.3%), unchanged in the rat (0.8%) and still not detected in human and dog.

At the high concentration 250 µM

No new metabolite was detected in any species at either an incubation time period of 4 or 24 hours. Transfluthrin was present in human incubates at a high level (45.2%) after the 4-hour incubation period. The parent compound was also detected at lower levels in two other species compared to the 25 µM concentration, i.e, 5.3% in the mouse and 4.2% in the rat.

After 4 hours of incubation, the levels of the major metabolite TFB alcohol in the rat, dog and mouse were higher than those measured at 25 µM for the same incubation period (4 hours) and represented 88.3%, 94.8% and 88.9%, respectively, of detected metabolites. In addition, the amounts of glucuronide-TFB alcohol in the same species were lower than those observed at the 25 µM concentration: 7.3 % for the rat, 5.1% for the dog and 4.5% for the mouse, which suggests a saturation of the biotransformation enzymatic system. No glucuronide of the TFB alcohol was detected in the human incubates. As seen previously, TFBA was present at very low levels in two species only, i.e., the rat and mouse.

After 24 hours of incubation, the parent compound was no longer detected in any species. The major metabolites were TFB alcohol for humans only (99.1%); TFB alcohol and its glucurono-conjugated metabolite for the rat (60.8% and 37.9%), dog (86.2% and 13.8%) and mouse (74.6% and 21.0%). The level of TFBA was slightly increased compared to the 4 hours incubation time and was still only in the rat (1.4%) and mouse (4.5%).

141.3.1 Reliability 1

141.3.2 Deficiencies No

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Mechanistic Study

Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2010
Materials and Methods	The version of the applicant is acceptable
Results and discussion	
Conclusion	RMS supports the conclusion.
Reliability	
Acceptability	Acceptable
Remarks	See doc IIIA 6.10 appendices with position papers mechanistic considerations dated 19-02-2010 and 10-05-2010. Furthermore the appendix interpretation of short-term assays regarding the effects of transfluthrin on rat urethelium in vivo and in vitro by [redacted] March 12, 2010. RMS supports the conclusions described in the position papers.
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.1/04-1. Authentic certified reference compounds used for metabolite identification

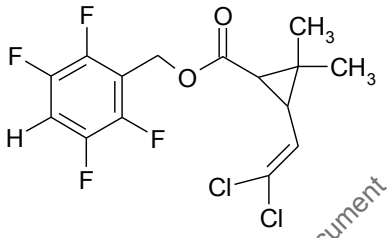
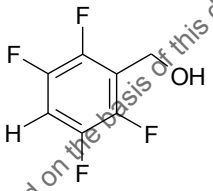
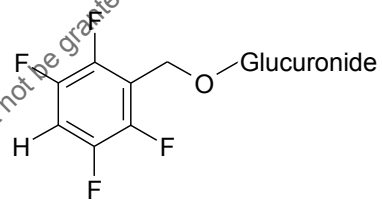
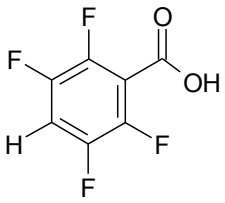
Certified References	Chemical Name (IUPAC)	Chemical Structure
active substance: Transfluthrin, (A0035474) Original sample ID: AE0035474 00 1B95 0001	2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate	
TFB-OH, (A1371431); TFB alcohol Original sample ID: M00225	2,3,5,6-tetrafluoro-benzylalcohol	
Glucoconjugated TFB alcohol, WAK5256 Original sample ID: WAK5256	Glucuronide conjugated of the 2,3,5,6-tetrafluoro-benzylalcohol	
TFBA; TFB acid, TFB-OOH, (FHW0119D) Original sample ID: 950627ELB01	2,3,5,6-tetrafluoro-benzoic acid	

Table A6.10/04-2. Distribution of the detected metabolites in the different samples expressed as relative percentage of the sum of the areas from the different peaks - At the low concentration

25 µM	Glucuronide-TFB alcohol	TFBA	TFB alcohol	Transfluthrin
Rt (min)	24.6-24.9	26.4-28.5	32.1-33.6	62.9-63.5
RAT				
4 hr	31.35	0.93	67.72	n.d.
24 hr	92.12	0.77	7.12	n.d.
HUMAN				
4 hr	0.79	n.d.	98.92	0.30
24 hr	5.66	n.d.	94.35	n.d.
DOG				
4 hr	34.07	n.d.	65.93	n.d.
24 hr	76.63	n.d.	23.38	n.d.
MOUSE				
4 hr	42.42	3.11	54.47	n.d.
24 hr	93.45	5.25	1.31	n.d.

n.d.: not detected

Table A6.10/04-3. Distribution of the detected metabolites in the different samples expressed as relative percentage of the sum of the areas from the different peaks - At the high concentration

250 µM	glucuronide-TFB alcohol	TFBA	TFB alcohol	Transfluthrin
Rt (min)	24.6-24.9	26.4-28.5	32.1-33.6	62.9-63.5
RAT				
4 hr	7.26	0.67	88.27	4.15
24 hr	37.88	1.37	60.76	n.d.
HUMAN				
4 hr	n.d.	n.d.	54.77	45.24
24 hr	0.93	n.d.	99.08	n.d.
DOG				
4 hr	5.13	n.d.	94.87	n.d.
24 hr	13.80	n.d.	86.21	n.d.
MOUSE				
4 hr	4.53	1.29	88.87	5.32
24 hr	21.00	4.46	74.55	n.d.

n.d.: not detected

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Mechanistic Study

The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

Official use only

142 REFERENCE

142.1 Reference

[redacted] (2010)

The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines, [redacted] **Study report No**: **299**, Report February 19, 2010 (unpublished).

BES Reference: Not yet allocated

142.2 Data protection Yes

142.2.1 Data owner: Bayer CropScience AG

142.2.2 Companies with letter of access: None

142.2.3 Criteria for data protection: Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

143 GUIDELINES AND QUALITY ASSURANCE

143.1 Guideline study No, no guidelines available

143.2 GLP No, but procedures were performed in accordance with SOPs that are in place at the laboratory. Various procedures, the protocol and final report were audited by a member of the Quality Assurance Unit from Bayer CropScience LP, Stilwell, Kansas.

143.3 Deviations No, there were no protocol deviations during the study

144 MATERIALS AND METHODS

144.1 Test material Transfluthrin

Tetrafluorobenzoic acid (TFBA)

144.1.1 Lot/Batch number: Transfluthrin: Batch No. ABIDTBN019; Certificate No. AZ 15885

Tetrafluorobenzoic acid: Batch No. 950627ELB01; Certificate No. AZ 12737

144.1.2 Specification: As given in section 2 for transfluthrin

144.1.2.1 Description: Transfluthrin: white melt

Tetrafluorobenzoic acid: white crystals

144.1.2.2 Purity: Transfluthrin: 99.6%

Tetrafluorobenzoic acid: 99.0%

144.1.2.3 Stability: Transfluthrin was stored at 25 ± 5ºC. Under these conditions transfluthrin is stable until April 30, 2011. Tetrafluorobenzoic acid was stored at 5 ± 5ºC. Under these conditions tetrafluorobenzoic acid is stable until June 21, 2013.

144.2 Preliminary test **cytotoxicity evaluation**

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Mechanistic Study

The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

144.2.1 Organism/cell type

MYP3 rat urothelial cell

1T1 human urothelial cell line

Provided by Dr. Ryoichi Oyasu, Northwestern University, Chicago, U.S.A.

144.2.2 Preparation

The MYP3 cell line was obtained from a small nodule that developed in a heterotopically-transplanted rat urinary bladder after treatment with N-methyl-N-nitrosourea (MNU). The cell line has retained the characteristics of epithelial cells in culture, expresses keratin 5 mRNA, does not exhibit anchorage-independent growth, and does not cause development of tumors in nude mice.

The 1T1 cell line was derived from benign ureter tissue obtained during a radical nephrectomy due to renal carcinoma in a 71-year old male. The cells were immortalized by transfection with the human papillomavirus type 16 E6 and E7 genes.

Cultural conditions

MYP3 cells were grown in Ham's F-12 medium (Gibco-BRL, Grand Island, NY) supplemented with 10 µM non-essential amino acids, 10 ng/ml EGF, 10 µg/ml insulin, 5 µg/ml transferrin, 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (all from Gibco) and 250 mg/ml dextrose and 1 mg/ml hydrocortisone from Sigma (St. Louis, MO).

1T1 cells were cultured in Keratinocyte-SFM (1x) with bovine pituitary extract (25 mg minimum), EGF (2.5 µg minimum) and 100 U/ml penicillin, and 100 µg/ml streptomycin (All from Gibco).

All cells were grown at 37° C in 5% CO_2.

144.2.3 Treatment

Each dose concentration was tested in 3 wells. MYP3 cells were seeded at a concentration of 1.0 x 10^4 cells/well in a 24-well plate. Since 1T1 cells grow more slowly than MYP3 cells, they were seeded at a concentration of 2.0 x 10^4 cells/well in a 24-well plate. Twenty-four hours later, treatment with each chemical was begun and continued for 3 days without changing the medium.

144.2.4 Dose preparation

A 1 M stock solution of transfluthrin was initially prepared in Dulbecco's PBS, 1x (Gibco). However, transfluthrin precipitated out of the solution. Attempts were made to solubilize lower concentrations of transfluthrin (50 and 100 mM) in absolute ethanol and dimethyl sulfoxide (DMSO), but were unsuccessful. Due to solubility problems with TFBA, it was determined that a 100 mM stock solution was the highest concentration of TFBA that could be solubilized. The stock solution was prepared by initially dissolving TFBA in absolute alcohol and then diluting to the final concentration of 100 mM with Dulbecco's PBS, 1x. Working solutions of TFBA were prepared in the medium appropriate for the cell line being used. All working concentrations of TFBA were mixed on the day treatment began and remained on the cells for 3 days.

144.2.5 Evaluation

Determination of cytotoxicity in vitro. To determine the range in which TFBA was cytotoxic to urothelial cells in culture, TFBA was first tested at 0.5, 1, 5, and 10 mM in MYP3 cells. TFBA was tested two more times over a narrow range of concentrations (Test 2-0.1, 0.25, 0.5, and 1 µM; Test

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3-0.5, 1, 2, and 3 µM) to confirm the LC_{50} (concentration at which the chemical is lethal to 50% of the cells) of TFBA for MYP3 cells. To determine the LC_{50} of TFBA for 1T1 cells, the concentrations of TFBA initially tested were 0.25, 0.5, 1, 2, 3, 5, and 10 mM. The LC_{50} was confirmed by testing TFBA at 1, 2, 3 and 5 mM.

Determination of cell viability and calculation of LC_{50}. Cell viability for each well was determined by staining with 0.4 % trypan blue (Sigma) and counting in a hemocytometer. The % survivability was calculated as the ratio of the mean cell number in the three treated wells to that in the control wells. The data were graphed with the known concentrations of the test material on the x axis and the % survivability at those concentrations on the y axis. The LC_{50} was calculated by linear regression analysis of the data using Microsoft Excel.

145 RESULTS AND DISCUSSION

Transfluthrin. Due to limitations of solubility, it was not possible to prepare a stock solution at a high enough concentration to determine the LC_{50} of transfluthrin for rat or human urothelial cells.

TFBA. The LC_{50} of TFBA for the rat urothelial cell line MYP3 was determined to be 2.25 mM (r^2=0.8564). The LC_{50} of TFBA for the human urothelial cell line 1T1 was determined to be 2.43 mM (r^2=0.8715).

146 APPLICANT'S SUMMARY AND CONCLUSION

146.1 Materials and methods

The *in vitro* effects of transfluthrin and tetrafluorobenzoic acid on rat and human urothelial cells lines were assessed.

The MYP3 rat urothelial cell line and the human urothelial cell line were used for cytotoxicity testing.

Cultural conditions

MYP3 cells were grown in Ham's F-12 medium (Gibco-BRL, Grand Island, NY) supplemented with 10 µM non-essential amino acids, 10 ng/ml EGF, 10 µg/ml insulin, 5 µg/ml transferrin, 10% fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin (all from Gibco) and 250 mg/ml dextrose and 1 mg/ml hydrocortisone from Sigma (St. Louis, MO).

1T1 cells were cultured in Keratinocyte-SFM (1x) with bovine pituitary extract (25 mg minimum), EGF (2.5 µg minimum) and 100 U/ml penicillin, and 100 µg/ml streptomycin (All from Gibco).

All cells were grown at 37° C in 5% CO_2.

Each dose concentration was tested in 3 wells. MYP3 cells were seeded at a concentration of 1.0 x 10^4 cells/well in a 24-well plate. Since 1T1 cells grow more slowly than MYP3 cells, they were seeded at a concentration of 2.0 x 10^4 cells/well in a 24-well plate. Twenty-four hours later, treatment with each chemical was begun and continued for 3 days without changing the medium.

A 1 M stock solution of transfluthrin was initially prepared in Dulbecco's PBS, 1x (Gibco). However, transfluthrin precipitated out of the solution.

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Mechanistic Study

The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

Attempts were made to solubilize lower concentrations of transfluthrin (50 and 100 mM) in absolute ethanol and dimethyl sulfoxide (DMSO), but were unsuccessful. Due to solubility problems with TFBA, it was determined that a 100 mM stock solution was the highest concentration of TFBA that could be solubilized. The stock solution was prepared by initially dissolving TFBA in absolute alcohol and then diluting to the final concentration of 100 mM with Dulbecco's PBS, 1x. Working solutions of TFBA were prepared in the medium appropriate for the cell line being used.

All working concentrations of TFBA were mixed on the day treatment began and remained on the cells for 3 days.

Determination of cytotoxicity in vitro. To determine the range in which TFBA was cytotoxic to urothelial cells in culture, TFBA was first tested at 0.5, 1, 5, and 10 mM in MYP3 cells. TFBA was tested two more times over a narrow range of concentrations (Test 2-0.1, 0.25, 0.5, and 1 µM; Test 3-0.5, 1, 2, and 3 µM) to confirm the LC_{50} (concentration at which the chemical is lethal to 50% of the cells) of TFBA for MYP3 cells. To determine the LC_{50} of TFBA for 1T1 cells, the concentrations of TFBA initially tested were 0.25, 0.5, 1, 2, 3, 5, and 10 mM. The LC_{50} was confirmed by testing TFBA at 1, 2, 3 and 5 mM.

Determination of cell viability and calculation of LC_{50}. Cell viability for each well was determined by staining with 0.4 % trypan blue (Sigma) and counting in a hemocytometer. The % survivability was calculated as the ratio of the mean cell number in the three treated wells to that in the control wells.

146.2 Results and Conclusion

Transfluthrin. Due to limitations of solubility, it was not possible to prepare a stock solution at a high enough concentration to determine the LC_{50} of transfluthrin for rat or human urothelial cells.

TFBA. The LC_{50} of TFBA for the rat urothelial cell line MYP3 was determined to be 2.25 mM (r^2=0.8564). The LC_{50} of TFBA for the human urothelial cell line 1T1 was determined to be 2.43 mM (r^2=0.8715).

146.3 Conclusion

146.3.1 Reliability: 1

146.3.2 Deficiencies: No

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Annex Point IIIA6.7

Mechanistic Study

The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	20 May 2010
Materials and Methods	The version of the applicant is acceptable
Results and discussion	
Conclusion	RMS supports the conclusion
Reliability	
Acceptability	Acceptable
Remarks	See doc IIIA 6.10 appendices with position papers mechanistic considerations dated 19-02-2010 and 10-05-2010. Furthermore the appendix interpretation of short-term assays regarding the effects of transfluthrin on rat urethelium in vivo and in vitro by [redacted] March 12, 2010. RMS supports the conclusions described in the position papers.
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Doc IIIA

Section 6.12.1

BPD Data Set IIA / Annex Point VI.6.9.1

Medical surveillance data on manufacturing plant personnel, if available

Official use only

147 REFERENCE

147.1 Reference

[redacted] (2006). Letter from W. Steffens re: Transfluthrin, dated 24.01.2006, [redacted].

[BES Ref: M-1-266138-01-1]
Non-GLP. Unpublished.

148 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

149 MATERIALS AND METHODS

Refer To Section 4 Below

150 RESULTS

Workers at three facilities have been exposed to transfluthrin during normal production, formulation, and testing of products. Standard workplace protection procedures include use of equipment to prevent dermal and respiratory exposure to fine particulates. Due to the lack of any workplace accidents, no significant acute human exposure is known to have occurred. Routine medical examinations of workers through 2004 have not produced clinical signs related to transfluthrin exposure. Therefore, there is no evidence to indicate that human response to transfluthrin exposure would differ significantly from responses seen in animal models.

151 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	30-05-2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	There is no evidence of negative health effects, signs or symptoms in people that work at sites where transfluthrin is produced or formulated.
Remarks	-

COMMENTS FROM ... *(specify)*

Date	*Give date of comments submitted*

Doc IIIA
Section 6.12.1

BPD Data Set IIA / Annex Point VI.6.9.1

Medical surveillance data on manufacturing plant personnel, if available

Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc III-A **SECTION 6.12.2** **BPD Data Set IIA/ Annex Point VI.6.9.2**	**Direct Observation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified [✓]** **Other existing data []** **Limited exposure [✓]**	
Detailed justification:	No known clinical cases of poisoning directly attributable to transfluthrin have been reported or are known to the Applicant.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30-05-2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable.	
Conclusion	No further data required.	
Remarks	-	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc III-A **SECTION 6.12.3** **BPD Data Set IIA/ Annex Point VI.6.9.3**	**Health Records**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified [✓]** **Other existing data []** **Limited exposure [✓]**	
Detailed justification:	See 6.12.1 (no other health records available to Applicant).	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30-05-2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable.	
Conclusion	No further data required.	
Remarks	-	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc III-A **SECTION 6.12.4** **BPD Data Set IIA/ Annex Point VI.6.9.4**	**Epidemiological Studies**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified []** **Other existing data []** **Limited exposure []**	
Detailed justification:	No epidemiological studies on transfluthrin have been conducted to the knowledge of the Applicant.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	30-05-2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable.	
Conclusion	No further data required	
Remarks	-	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA — Diagnosis of poisoning including specific signs of poisoning and clinical tests, if applicable

SECTION 6.12.5

BPD Data Set IIA / Annex Point VI.6.9.5

Official use only

152 REFERENCE

152.1 Reference

[redacted] (2006). Transfluthrin. Medical information. Poisoning – diagnosis, treatment and prognosis. Bayer Environmental Sciences. Lyon, France.

[BES Ref: M-266732-01-1]
Non-GLP. Unpublished.

153 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

154 MATERIALS AND METHODS

Refer To Section 4 Below

155 RESULTS

In cases of contact to pyrethroids the first sign of exposure is a specific paresthesia/irritation, often described as "cold burn". This may appear immediately or shortly after contact to the substance, or may last up to 24 (rarely to 48) hours and often is reported to be worsened by warmth (e.g. showering). This "cold burn" is due to a stimulation of free nerve endings, and is dependant on concentration, not on dose. It is strictly a local symptom only and not a symptom of a general poisoning. The irritation can occur both on the skin and on the mucous membranes of the airways. In the latter case in sensible individuals an asthma-like non-specific response can be triggered.

In case of severe intoxication non-alpha-cyano pyrethroids may cause the following signs and symptoms as seen in animal experiments and suicidal poisoning cases:

Organ (system)	Signs/symptoms	Remarks
Skin	paresthesia/irritation ("cold burn")	local only
Mucous membranes	irritation, cough, sneezing	local only
Lung	chest tightness, airway hyper-reaction, pulmonary edema	
Heart/circulation	tachycardia, hypotension, palpitations	
Gastrointestinal tract	nausea, vomiting, diarrhea, abdominal pain	
Central Nervous System	dizziness, blurred vision, headache, listlessness, anorexia,	

Doc IIIA — Diagnosis of poisoning including specific signs of poisoning and clinical tests, if applicable

SECTION 6.12.5

BPD Data Set IIA / Annex Point VI.6.9.5

	somnolence/coma, seizures/convulsions; tremor, prostration	

156 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5 June 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	The version of the applicant is adopted
Remarks	-
	COMMENTS FROM … *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc III-A **SECTION 6.12.6** **BPD Data Set IIA/ Annex Point VI.6.9.6**	**Sensitisation/Allergenicity Observations**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified []** **Other existing data []** **Limited exposure []**	
Detailed justification:	No sensitizations have been described within BCS or in the literature.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	5 june 2007	
Evaluation of applicant's justification	A quick scan of literature thus not reveal evidence for sensitizing properties of transfluthrin.	
Conclusion	The justification of the applicant is acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc IIIA Specific treatment in case of accident or poisoning

SECTION 6.12.7

BPD Data Set IIA / Annex Point VI.6.9.7

Official use only

157 REFERENCE

157.1 Reference

[redacted] (2006). Transfluthrin. Medical information. Poisoning – diagnosis, treatment and prognosis. Bayer Environmental Sciences. Lyon, France.

[BES Ref: M-266732-01-1]
Non-GLP. Unpublished.

158 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

159 MATERIALS AND METHODS

Refer To Section 4 Below

160 RESULTS

First Aid:

- Remove patient from exposure/terminate exposure

- Thorough skin decontamination with water and copious amounts of detergents/soap - pyrethroids are only little soluble in water.

Note: Warm water may increase the subjective severity of the irritation/paresthesia, which is not sign of systemic poisoning.

- Flushing of the eyes with lukewarm water for 15 minutes, apply soothing eye-drops, if needed anaesthetising eye-drops.

- 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 not recommended, if a formulation containing organic solvents has been ingested (aspiration hazard).

Treatment:

Gastric lavage should be considered in cases of ingestion of high doses within the first (2) hour(s). However, the application of activated charcoal and sodium sulphate is always advisable in significant

Doc IIIA Specific treatment in case of accident or poisoning

SECTION 6.12.7

BPD Data Set IIA / Annex Point VI.6.9.7

ingestions.

There is no specific antidote for pyrethroids, so any treatment thus can only be symptomatic.

Reports from the USA seem to indicate a positive effect of vitamin-E-containing oils on the irritation/paresthesia; however, there is no real proof of this. The skin application of oils or lotions containing vitamin E may be considered. The skin irritation may be painful and require the application of analgesics; anesthetic eye-drops 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.

161 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

Evaluation by Competent Authorities

EVALUATION BY RAPPORTEUR MEMBER STATE

Date	5 June 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	The version of the applicant is adopted
Remarks	-

COMMENTS FROM ... *(specify)*

Doc IIIA Specific treatment in case of accident or poisoning

SECTION 6.12.7

BPD Data Set IIA /
Annex Point VI.6.9.7

Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc IIIA Prognosis following poisoning

SECTION 6.12.8

BPD Data Set IIA / Annex Point VI.6.9.8

Official use only

162 REFERENCE

162.1 Reference

[redacted] (2006). Transfluthrin. Medical information. Poisoning – diagnosis, treatment and prognosis. Bayer Environmental Sciences. Lyon, France.

[BES Ref: M-266732-01-1]
Non-GLP. Unpublished.

163 GUIDELINES AND QUALITY ASSURANCE (NOT APPLICABLE)

164 MATERIALS AND METHODS

Refer To Section 4 Below

165 RESULTS

Recovery is spontaneous and without sequelae.

166 APPLICANT'S SUMMARY AND CONCLUSION

Refer to section 4 above

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5 June 2007
Materials and Methods	The version of the applicant is acceptable.
Results and discussion	The version of the applicant is adopted
Conclusion	The version of the applicant is adopted
Remarks	-
	COMMENTS FROM ... *(specify)*
Date	*Give date of comments submitted*
Materials and Methods	*Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*

Doc IIIA Prognosis following poisoning

SECTION 6.12.8

BPD Data Set IIA /
Annex Point VI.6.9.8

Remarks

Doc III-A SECTION 6.13 **BPD Data Set IIA/ Annex Point IIIA VI.2**	**Toxic effects on livestock and pets**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified [✓]** **Other existing data []** **Limited exposure [✓]**	
Detailed justification:	Pyrethroids have a long history of direct use in veterinary medicines to control parasites such as fleas and ticks. These formulations are applied directly to the fur/coat of the animal to control the pest at a local level. The following pyrethroids have approval for veterinary use: Flumethrin: For treatment of sheep using a 6% EC solution Cattle dip and cattle tick spray at 75 g flumethrin/l Pour-on solution for cattle tick at 10g/l Deltamethrin: Dip, spray at 50g/l Pour-on at 7.5g/l (Source: www.fao.org) Transfluthrin is to be formulated in three different products which are all for amateur use. Baygon Mosquito Coil is a mosquito coil which, on burning, releases the active substance as a vapour over a period of 8 hours. Raid Portable Electric is a vapouriser which releases transfluthrin from an impregnated mat and is recommended for use over an 8 hour period. Turbo 4 Seasons is also an impregnated disc which is used in wardrobes and closets to control moths and releases transfluthrin by volatilisation over a period of 3 months. Baygon Mosquito Coil is the only product which is recommended for use indoors and outdoors. None of the products are recommended for use specifically in spaces where animals such as livestock are housed. The levels of exposure associated with the other pyrethroid products listed above far exceed the potential exposures associated with the use of the transfluthrin products. Toxicity testing is by principle conducted on several species and key reference values based on NOELs in the most sensitive of those species. Toxicology NOELs will therefore be in principle protective of most species of household pets.	

Doc III-A SECTION 6.13 **BPD Data Set IIA/ Annex Point IIIA VI.2**	**Toxic effects on livestock and pets**
	According to the TNsG for human exposure, the only exposure scenario of interest for strips or cassettes placed in closed spaces is inhalation exposure associated with use of the product. Consequently, there is no significant post-application exposure to pets or livestock which needs to be taken into consideration (i.e. from dislodgeable condensed residues). There may be very limited, almost negligible, inhalation exposure to pets that inhabit the same residential space as their owners, therefore for completeness a basic assessment has been conducted for potential exposure to a dog (i.e. typical household pet). The highest mean event air concentration for the three products predicted by CONSEXPO (version 3.0) is 0.0154 mg/m^3 for Turbo 4 Seasons (refer to Turbo 4 Seasons Document IIB, Section 3.2.3.1). This is for concentrations of transfluthrin in the treated wardrobe or closet and therefore represents a worst case as pets would have to enter the wardrobe to achieve these levels of exposure. Uptake of airborne residues would be via inhalation of airborne transfluthrin for this basic assessment it is assumed that there is 100% retention and absorption of inhaled material. Secondary dermal exposure is not relevant as explained above and furthermore a protective coat or fur would also prevent penetration. Based upon a breathing rate of 4.5 litres/minute ("Animal Models in Toxicology", Gad and Chengelis – Library of Congress Cataloging in Publication, 1992) and a typical bodyweight of 10kg for dogs, the following exposures have been calculated: Inhalation rate = 4.5 l/min (0.27 m^3/hr) Exposure: 0.0154 mg/m^3 x 8 hr/day x 0.27 m^3/hr x 100 % absorption and retention x 1/10 kg bw = 0.0033 mg/kg bw/day For short term inhalation exposure, the most appropriate end point is the NOAEL of 16.8 mg kg/bw day from the 13-wk inhalation study in the rat (see Document IIA Section 3.5 for summary of relevant toxicology end points). Comparion of the exposure to this end point gives a TER of > 5000, i.e. a high margin of safety. Therefore there is very low potential risk to domestic pets through use of the transfluthrin products.
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5 June 2007

Doc III-A SECTION 6.13 BPD Data Set IIA/ Annex Point IIIA VI.2	Toxic effects on livestock and pets
Evaluation of applicant's justification	The scenario of a dog sitting in the wardrobe for 8 hours is unrealistic worst case. However, as that still does not lead to potential risk, the applicant's justification is acceptable.
Conclusion	The justification of the applicant is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Doc III-A **SECTION 6.14** **BPD Data Set IIA/ Annex Point IIA. XI.2**	**Other tests relating to the exposure of humans**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
	Technically not feasible [] **Scientifically unjustified [✔]** **Other existing data []** **Limited exposure []**	
Detailed justification:	No additional tests relating to the exposure of humans are available. There are no toxicologically relevant degradation products, by-products or reaction products of transfluthrin which could be considered as requiring additional testing. Therefore, this data requirement is considered not to be applicable to transfluthrin.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	5 June 2007	
Evaluation of applicant's justification	The justification of the applicant is acceptable.	
Conclusion	The justification of the applicant is acceptable.	
Remarks	-	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	
Date	*Give date of comments submitted*	
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*	
Conclusion	*Discuss if deviating from view of rapporteur member state*	
Remarks		

Doc III-A **SECTION 6.15** **BPD Data Set IIIA/ Annex Point VI.4** **XI.1.1, 1.2, 1.3, 1.5, 1.6**	**Food and feedingstuffs: Identification of degradation and reaction products and of metabolites, behaviour of the residue of the active substance its degradation products and metabolites, including the kinetics of disappearance, in treated or contaminated foods or feedstuffs.**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] **Limited exposure [✓]**	**Technically not feasible [ ]** **Scientifically unjustified [✓]** **Other justification [ ]**	
Detailed justification:	Baygon Mosquito Coil containing 0.03% transfluthrin is formulated in a ready-to-use Mosquito Coil to be used by non-professionals, both indoors and outdoors. On burning, the active substance is released from the coil as a vapour over a period of 8 hours. Subsequent environmental exposure is therefore to the active substance only. Raid Portable Electric containing 220 mg (*ca* 18.8%) transfluthrin is formulated in a ready-to-use vapouriser to be applied by non-professionals, indoors only. Use will be seasonal to some extent and applications are localised. The product is designed to treat room sizes of up to 20m^2 and is recommended for use for up to 8 hours in any one day. Subsequent environmental exposure is therefore to the active substance only. Turbo 4 Seasons containing 7.5 mg transfluthrin is formulated as a ready-to-use household product to be applied by non-professionals (indoors only). The product is designed to treat cupboards, closets and wardrobes. The discs release transfluthrin into the air through a process of volatilisation. However, according to the TNsG, the only exposure scenario of interest for strips or cassettes placed in closed spaces is inhalation exposure associated with use of the product. Consequently, there is no post-application human exposure (i.e. from dislodgeable condensed residues) for the Turbo 4 Seasons Anti-Moth product (Document II-B, section 3.2.4). On this basis, a consideration of post application environmental exposure from dislodgeable residues is similarly not considered relevant. The estimation of potential exposure of the active substance to humans through diet and other means has been carried out (Documents IIB-1 and IIB-2, section 3.2), taking into account the frequency and duration of use, the emission rate of the active substance from the product, assuming that the airborne fraction of emitted residues is 100% and using standard room volume and ventilation rates. The mean event indoor air concentration was higher from the use of the vapouriser than from the coil and therefore subsequent worst case calculations are presented for Raid Portable Electric only. The mean event indoor air concentration was predicted to be 0.00735 mg/m^3 and an estimation of residues deposited on surfaces of a treated room was carried out. The total dislodgeable transfluthrin residue following 150 days of use, assuming a default 30% dislodgeable fraction (Document II-B, section 3.2.4), was estimated to be 0.00756 mg/m^2. However, assuming that 100% of residues are dislodgeable (are transferable), and on a *daily* basis, the total residue would be 0.17 µg/m^2 (1.68 x 10^{-4} mg/m^2). This is	X

equivalent to 0.017 ng/cm^2.

(a) Assuming that this residue deposited on a table surface on which a sandwich was then placed, and that 100 % of the residue on the table surface were dislodged and transferred to the sandwich (lower surface area of sandwich = 150 cm^2[17]), the intake would be:

for a 10 kg toddler: $\frac{0.017 \text{ ng cm}^{-2} \times 150 \text{ cm}^2}{10 \text{ kg}} = 0.26 \text{ ng kg}^{-1}$

= 2.6 x 10^{-7} mg active substance per kg body weight, per day.

for a 60 kg adult : $\frac{0.017 \text{ ng cm}^{-2} \times 150 \text{ cm}^2}{60 \text{ kg}} = 0.04 \text{ ng kg}^{-1}$

= 4.25 x 10^{-8} mg active substance per kg body weight, per day.

(b) Assuming that a sandwich, which had received this dislodged residue from the table surface, was also to receive direct deposition of the active substance onto its upper surface (upper surface area of sandwich = 150 cm^2). Then, the intakes would be two times those calculated in (a) above:

for a 10 kg toddler: 5.2 x 10^{-7} mg/kg bw/day

for a 60 kg adult: 8.5 x 10^{-8} mg/kg bw/day

(c) An extreme worst case scenario has also been considered for comparison. This assumes that no cleaning of the surface takes place at all during the 150 days duration of product use and that a sandwich placed on the surface on day 150 receives 149 days worth of 100% dislodged residues from this surface and direct deposition of the active substance onto its upper surface over 1 day. The intakes would be:

for a 10 kg toddler: [(2.6 x 10^{-7} x 149) + 2.6 x 10^{-7}] = 3.9 x 10^{-5} mg/kg bw/day

for a 60 kg adult: [(4.25 x 10^{-8} x 149) + 4.25 x 10^{-8}] = 6.4 x 10^{-6} mg/kg bw/day

Chronic exposure: A comparison of estimated intakes from potential contaminated food with the proposed ADI (Acceptable Daily Intake) of 0.01 mg/kg bw/day (Document III-A, section 6.7/01, Document II-A section 3) for scenarios (b) and (c) shows that respective adult and toddler intakes are 0.0009 – 0.064% and 0.005 – 0.39% of the ADI (from the consumption of one sandwich). An adult would need to consume 1,562 – 117,647 sandwiches per day and a toddler would need to consume 256 – 19,231 sandwich es per day to achieve intakes equivalent to the ADI.

Acute Exposure: A comparison of estimated intakes from potential contaminated food with the proposed ARfD (Acute Reference Dose) of 0.17 mg/kg bw/day (Document III-A, section 6.4.3, Document II-A section 3) for scenarios (b) and (c) shows that respective adult and toddler intakes are 0.00005 – 0.004% and 0.0003 – 0.02% of the ARfD of 0.5 mg/kg bw/day. In order to achieve the acute reference dose, an adult would need to consume 26,562 – 2,000,000 sandwiches per day and a toddler would need to consume 4359 – 326,923 sandwiches per day.

It is unlikely that food to be consumed would be left uncovered on a work surface for a whole day prior to consumption and highly unlikely that a work surface would not be cleaned for 150 days. Even assuming these worst case scenarios, a toddler or adult

[17] UK HSE, *Pers. Comm*.

	could not eat this number of sandwiches in a day and therefore the risk to consumers from the consumption of potentially contaminated food from the proposed use of the vapourisr is considered to be acceptable. In summary, the amateur indoor use of transfluthrin in the vapouriser, with subsequent deposition and transference of residues from room surfaces to foodstuffs (sandwich of 150 cm^2 surface area), results in negligible potential residue levels in food which do not pose a risk to consumers. The need to conduct studies on residues in food and feedingstuffs is therefore considered to be scientifically unjustified.
Undertaking of intended data submission [ ]	Not applicable
	Evaluation by Competent Authorities
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	19 June 2007
Evaluation of applicant's justification	The mean event indoor air concentration was predicted to be 0.038 mg/m^3, which is 5 times higher than the 0.00735 mg/m^3 stated by the notifier. Results of all calculations will end this factor 5 higher. However, the conclusion will remain the same.
Conclusion	There is no need to conduct studies on residues of the biocidal product in food as calculations show that potential residue levels in food will be negligible.
Remarks	-
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	
Evaluation of applicant's justification	
Conclusion	
Remarks	