Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

Transfluthrin (ISO); 2,3,5,6-tetrafluorobenzyl (1*R*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

EC Number: 405-060-5 (ELINCS)*

CAS Number: 118712-89-3*

Index Number: 607-223-00-8 *

** The EU index no. and ELINCS no. refer to the 1R,trans and 1S,trans configurations, which is not in agreement with the definition of transfluthrin, which is exclusively the 1R,trans isomer. The CAS registry no. does refer to the correct isomer.*

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Version number: 2.0 **Date: October 2019**

CONTENTS

1 PHYSICAL HAZARDS

1.1 Explosives

Study 1 reference: Smeykal (2005), M-251690-01-1

Test type
The purpose of this study was the determination of the explosive properties of the test item according to the consolidated version of Council Directive 67/548/EEC Annex V, Part A: Methods for the determination of physico-chemical properties.A. 14. Explosive properties and OECD test guideline, OECD 113 Screening test for thermal stability and stability in air. The study is performed under GLP.

Material and methods
Transfluthrin (Bayothrin), NAK 4455; Substance, technical (purity 99.1 % (w/w)) was tested.

Thermal Stability
As a preliminary measurement the determination of the thermal stability was carried out by differential scanning calorimetry (DSC). The test item and the standard material (aluminium oxide) were heated up from ambient temperature to the final temperature (450 °C) at a constant heating rate (3 K/min) in a defined atmosphere (nitrogen). Closed glass crucibles were used as containers for the test item and the standard material. When stainless steel crucibles were used the standard crucible was empty. In this case the crucibles were heated up to a final temperature of 500 °C. The quantity of heat absorbed or released was measured and recorded. Amounts of samples of the test item of about 11 -17 mg were used.

Explosive Properties
As a screening method for the determination of explosive properties a differential scanning calorimetry (DSC) under nitrogen was performed. If the decomposition energy is below 500 J/g a main test for explosive properties is unnecessary (Recommendations on the Transport of Dangerous Goods / Manual of Tests and Criteria (ST/SG/AC.10/11/Rev.3), page 398). The method provides a scheme of testing to determine whether or not a solid, a liquid or a pasty substance or preparation presents a danger of explosion when submitted to the effect of a flame (thermal sensitivity) or to impact or friction (sensitivity to mechanical stimuli).
The method comprises three parts:

a) a test of thermal sensitivity;
b) a test of mechanical sensitivity with respect to shock;
c) a test of mechanical sensitivity with respect to friction.

Thermal Sensitivity
The method involves heating the substance or preparation in a steel tube, with various degrees of confinement being provided by nozzle-plates with a diameter (2 mm) of orifice, to determine whether the substance or preparation is liable to explode under conditions of thermal stress.
Mechanical Sensitivity (Shock)
The method involves subjecting the substance or preparation to the shock of a falling hammer on a steel anvil.
Mechanical Sensitivity (Friction)
The method involves subjecting the substance or preparation to friction between standard surfaces

under specified conditions of load and relative motion. This test is restricted to solid and pasty substances or preparations.

Results
Thermal Stability The DSC-measurement in the closed glass crucible showed an endothermic effect (melting) at a temperature of about 30 °C and an exothermal decomposition in the temperature range 250 - 390 °C with an energy of 420 J/g. Due to the high pressure in the crucible caused by the evaporation of the test item accompanied with the risk of the burst of the crucible the measurement could not be carried out up to 500 °C, it had to be stopped at 450 °C.
An additional run in a closed stainless steel crucible was performed. The measurement showed an endothermic effect (melting) at a temperature of about 30 °C and an exothermal decomposition in the temperature range 250 - 390 °C with an energy of 618 J/g.

Explosive Properties
The heat of decomposition was above 500 J/g. Therefore the main test on explosion properties was performed.
Mechanical Sensitivity (Friction):
No explosion was observed within 6 tests using a pinload with 360 N.
Mechanical Sensitivity (Shock):
No explosion within 6 tests using a mass of 10 kg falling from a height 0.4 m was observed. After these tests the colour of the test item changed to light brown due to a decomposition of the test item.
Thermal sensitivity:
The test item was liquefied, before the measurement. Three tests were performed with a circular nozzle plate with a hole of 2 rnm diameter.

Test no.	Weight of test item (g)	Nozzle width (mm)	T1 (s)	T2 (s)	reaction	Appearance; number of fragments
1	39.1	2.0	45.1	> 300.0	Flame	0
2	38.5	2.0	52.5	> 300.0	Flame	0
3	39.0	2.0	52.7	> 300.0	Flame	0

ti: time to any reaction, e.g. whistle or flame
t2: time to any reaction and duration of reaction, e.g. rupture of tube or flashing

The test on thermal sensitivity carried out with a nozzle with a diameter of 2.0 mm showed no explosion. Compared to the nozzle with a diameter of 6.0 mm the venting area of the nozzle with 2.0 mm is smaller by an order of magnitude. Additionally the physical state of the test item (pure substance) excludes the enrichment of high-energy components caused by evaporation of volatile solvents. Due to these facts an explosion with a nozzle width of 6.0 mm is excluded.

1.2 Flammable gases (including chemically unstable gases)
Not relevant.

1.3 Oxidising gases
Not relevant.

1.4 Gases under pressure
Not relevant.

1.5 Flammable liquid

Not relevant.

1.6 Flammable solids

1.6.1 Study 1 – flash point

Study 1 reference: Smeykal (2005), M-254399-01-1

Test type

The purpose of this study was the determination of the flash point of the test item according to the consolidated version of Council Directive 67/548/EEC Annex V, Part A: Methods for the determination of physico-chemical properties. A.9. Flash point: ISO 3679, ISO 3680: Setaflas. The study is performed under GLP.

Material and methods

Transfluthrin (Bayothrin), NAK 4455; Substance, technical (purity 99.1 % (w/w)) was tested.

The flash point is the lowest temperature, corrected to a pressure of 1013.3 hPa, at which the test liquid in a closed test vessel evolves vapour, under the conditions defined in the test method, in such amount that a flammable vapour/air mixture is produced in the test vessel. The flash point indication is carried out visually, and automatically.

The flash point is corrected to a pressure of 1013 hPa and the average is formed.

Flp. = 0 + 0.025(1013-p)

Flp.: flash point corrected to 1013 hPa in °C

C: measured flash point in °C

p: ambient pressure in hPa

According to the guideline the temperature for the final flash point is rounded down with an accuracy of 0.5 °C.

Setaflash:

As a preliminary measurement the test item was placed in the test vessel at the starting temperature of 170 °C, 150 °C, 120 °C and subsequently the test vessel was progressively heated (2 K/min) until the vapour reaches a sufficiently high concentration in air to produce a flammable mixture which could be ignited. If no ignition was observed the apparatus stopped 30 K above the starting temperature. In this preliminary test the flame was periodically (every 60 sec) dipped into the gas atmosphere of the test vessel. The test vessel was filled with 4 cm3 of the test item. Whereas the preliminary measurement was carried out at a heating rate of 2 K/min, the main test was at each time performed at constant temperature and equilibrium conditions. After the vessel was heated up to the starting temperature a sample of the test item was transferred into the apparatus. The ignition of the test item by digging in the flame was carried out after the test item was held for a minimum of 60 seconds at constant temperature.

Results

Preliminary measurement:

Volume / ml	Start temperature / °C	Heating rate / K/min	Measured flash point / °C

4	170	2	172
4	150	2	152
4	120	2	124

Main Test:
The following table shows the results of the main test, and temperatures, where ignition was detected (+) and where not (-).

Volume / ml	Temperature /°C	Ignition + / -	Barometric pressure / hPa	Flashpoint / °C
4	124.0	+		(corrected)
4	119.0	-		—
4	120.0	-		—
4	121.0	+		—
4	120.0	-		—
4	120.0	+		—
4	119.0	-		—
4	119.5	+		—
4	119.0	+	993.2	—
4	118.0	-		119.495
4	118.5	-		—
4	118.5	-		—
4	118.5	-		—
4	118.5	-		—
4	119.0	+		—
4	119.0	+		119.495
4	119.0	+		119.495

Flash point corrected to 1013 hPa: 119.0 °C.

1.6.2 Study 2 – melting point

Study 1 reference: Smeykal (2005), M-254400-01-1

Test type
The purpose of this study was the determination of the melting point and the thermal stability of the test item according to the consolidated version of Council Directive 67/548/EEC Annex V, Part A: Methods for the determination of physico-chemical properties. A.1. Melting point / melting range and OECD test guidelines, OECD 102 Melting point / melting range OECD 113 Screening test for thermal stability and stability in air. The test is performed under GLP.

Material and methods
Transfluthrin (Bayothrin), NAK 4455; Substance, technical (purity 99.1 %(w/w)) was tested.

Thermal Stability : Differential Scanning Calorimetry
The determination of the thermal stability was carried out by differential scanning calorimetry (DSC). The test item and the standard material (aluminum oxide) in closed glass crucibles were heated up from ambient temperature to the final temperature (450 °C) at a constant heating rate (3 K/min) in a defined atmosphere (nitrogen). When stainless steel crucibles were used the standard crucible was empty. In this case the crucibles were heated up to a final temperature of 500 °C.

The quantity of heat absorbed or released was measured and recorded. Amounts of samples of the test item of about 11 - 17 mg were used (see project 20050216.03).

Melting Point / Melting Range: Differential Scanning Calorimetry
The determination of the melting and the boiling point was carried out by differential scanning calorimetry (DSC). The test item was heated up from -20 °C to the final temperature (100 °C) at a constant heating rate (10 K/min) in a defined atmosphere (nitrogen). The quantity of heat absorbed or released was measured and recorded. Aluminium crucibles with a small hole were used as containers for the test item and as empty reference crucible. Amounts of samples of the test item of about 17-21 mg were used.

Results
Thermal Stability (DSC)
The DSC-measurement in a closed glass crucible showed an endothermic effect (melting) at a temperature of about 30 °C and an exothermal decomposition in the temperature range 250 - 390 °C with an energy of 420 J/g. An additional run in a closed stainless steel crucible was performed. The measurement showed an endothermic effect (melting) at a temperature of about 30 °C and an exothermal decomposition in the temperature range 250 - 390 °C with an energy of 618 J/g. But due to the high pressure in the crucible, caused by the vapour pressure of volatile components of the test item, the crucible was leaking at approx. 497 °C.
Melting Point / Melting Range (DSC)
Two DSC-runs in aluminium crucibles with a hole showed an endothermic effect (melting) in the temperature range 20 - 45 °C with an onset temperature of 32 °C.

Ident-No.	Onset / °C	Temperature range / °C	Crucible
21377	31.62	20-45	Aluminium with a hole
21381	31.88	20-45	Aluminium with a hole

The melting point of the test item is 32 °C.

1.6.3 Study 3 – flash point and auto-ignition

Study 1 reference: Heitkamp (2001), M0-03-010048

Test type
The tests were carried out following guidelines Guideline 92/69/EWG (German), 'Methods for Determination of Physicochemical Properties', Official gazette No L 383 A of December 1992, A-9 Flash point, A-15 Spontaneous Combustion Determination of the Auto Ignition Temperature of Liquids and Gases. The study is performed under GLP.

Material and methods
Transfluthrin (purity 95.7%) was tested.

Determination of the Flash Point (EC A 9)
The test was performed as stipulated in EC Test Procedure A 9 and the Bayer SOP (Standard Operation Procedure) for flash point determination.
The tests were performed as stipulated in DIN EN 456. A gas flame was used as the ignition source. Ignitions were visually determined by the tester.

Spontaneous Combustion Testing (Determination of the Auto Ignition Temperature of Liquids and Gases - EC A 15)

The test was performed as stipulated in Test Procedure A 15 and DIN 51794. Narrow-neck Erlenmeyer flasks with a nominal volume of 200 rnL were used as ignition vessels. A 'Eurotherm' regulating system was used to control the temperature of the electric furnace. Different sample sizes were added for the individual measurements. Testing commenced with a range-finding determination of the auto ignition temperature.

Results

Determination of the Flash Point (EC A 9)

In the first and second main tests, ignitions were determined at 122.3°C. The barometric pressure was 1002 hPa at the time of the test runs. Corrections with respect to the barometric pressure resultet in a final value of 122°C. 'NAK 4455 techn. (Bayothrin)' exhibits a flash point of 122°C according to DIN EN 456.

Spontaneous Combustion Testing

An initial ignition was observed at a temperature of 420°C. Proceeding from this temperature, the lowest result in the main test run was found to be 415°C. A repeat test was performed using one fresh ignition vessel. The lowest result in the repeat test was 415°C, too. Based on the individual measurements, the auto ignition temperature of the 'NAK 4455 techn. (Bayothrin)' test substance is specified as 415°C.

1.7 Self-reactive substances

No data available.

1.8 Pyrophoric liquids

Not relevant.

1.9 Pyrophoric solid

No data available.

1.10 Self-heating substances

Please refer to 1.6.3, Study 3 – flash point and auto-ignition.

1.11 Substances which in contact with water emit flammable gases

No data available.

1.12 Oxidising liquids

Not relevant.

1.13 Oxidising solids

Study 1 reference: Smeykal, H (2006), M-268646-01-1

Test type

The study is performed under GLP.

Material and methods

Transfluthrin (Bayothrin), NAK 4455; Substance, technical (purity 99.1%) was tested.

The test method A.21 for the oxidising properties of liquids and not the test method A.17 for the oxidizing properties of solids was used, due to the fact that the test item melts already at a

temperature of 32°C and therefore it was not possible to grind the test item to a particle size < 125 µm.
The test method is designed to measure the potential for a liquid substance to increase the burning rate or burning intensity of a combustible substance when the two are thoroughly mixed or to form a mixture which spontaneously ignities. The liquid is a mixture in a 1 to 1 ratio, by mass with fibrous cellulose, the mixture is heated in a pressure vessel and the rate of pressure rise is determined. Mixtures of oxidizers with cellulose must be tertaed as potentially explosive and handeled with care.

Results
A test series of at least 5 tests each were performed with the test item and with 65% nitric acid in 1:1 mixture, by mass, with cellulose.
Reference item: In table 1.13-1 the pressure rise times from 690 kPa_g to 2070 kPa_g for the 1:1 mixtures, by mass, of 65% nitric acid and cellulose are shown.

Table 1.13-1: pressure rise time for the 1;1 mixtures, by mass, of 65% nitric acid and cellulose

	Test 1	Test 2	Test 3	Test 4	Test 5
Time at 690 kPAg in s	9.69	10.20	10.98	10.92	9.67
Time at 2070 kPAg in s	13.23	13.73	15.17	14.61	14.31
Pressure rise time in s	3.54	3.53	4.19	3.69	4.64

The mean pressure rise time is 3.91s. All measured values are within the tolerable range of ±30% (2.74-5.09s) of the mean pressure rise time.
Test item: in table 1.13-2 the pressure rise times from 690 kPa_g to 2070 kPa_g for the 1:1 mixtures, by mass of the test item and cellulose are shown.

Table 1.13-2: pressure rise time for the 1;1 mixtures, by mass, of 65% nitric acid and cellulose

	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test 7
Time at 690 kPAg in s	14.63	12.72	16.05	15.74	15.03	15.50	13.87
Time at 2070 kPAg in s	31.67	25.68	-	-	-	-	-
Pressure rise time in s	17.04	12.96	-	-	-	-	-

Only in two cases the bursting pressure of the rupture disc of 2200 kPa was reached. In the other 5 measurements the pressure did not rise up to bursting pressure of the rupture disc.

Due to the fact that the 1”1 mixture, by mass, of test item and cellulose has a mean pressure rise time higher than that of a 1:1 mixture, by mass, of 65% nitric acid and cellulose or that the 1:1 mixture, by mass, of test item and cellulose did not reach the bursting pressure of the rupture disc at all, the test item has no oxidizing properties.

This result is also proven by an evaluation of the chemical structures of the test item. The test item contains oxygen, chloride and fluorine, but these elements are chemically bonded only to carbon. It is known that these structures do not have any oxidizing properties.

1.14 Organic peroxides

Not relevant.

1.15 Corrosive to metals

Not relevant.

1.16 Other studies

Reference	Eyrich, U, 2017 Study title: Transfluthrin (EC 0035474): Partition coefficients 1-octanol / water at pH 4, pH 7 and pH 9 (HPLC method)	**Official use only**
Data protection	Yes	
Data owner	Bayer AG	
Guideline study	OECD 117 / EC A.24 (HPLC method)	
GLP	Yes, study ID PA17/015	
Deviations	None reported	
Materials and methods	The material used was transfluthrin (2,3,5,6-tetrafluorobenzyl (1R,3S)-3-(2,2-dichlorovinyl)-2,2-Dimethylcyclopropanecarboxylate) with a purity of 99.1%. The partition coefficients of transfluthrin was determined using the HPLC method at 25°C. A calibration curve was made using retention times of the following reference compounds : acetanilide (log Pow 1.0), cinnamyl alcohol (log Pow 1.9), 2,6-dichlorobenzonitrile (log Pow 2.6), allyl phenyl ether (log Pow 2.9), benzophenone (log Pow3.2), cumene (log Pow 3.7), diphenyl ether (log Pow 4.2), dibenzyl (log Pow 4.8), fluoranthene (log Pow 5.1) and DDT (log Pow 6.5) in accordance with OECD guideline 117 (April 2004). Dead time of the HPLC system was determined using thiourea injections. HPLC conditions: Column: 50mmx4.6mm, C18 stationary phase (1.8µm), 25°C Mobile phase: acetonitrile / 0.01 mol/L phosphoric acid, adjusted to pH 4, pH 7 and pH 9 with sodium hydroxide 70/30, isocratic elution. UV detection at 210nm (thiourea and test item), 254nm, (calibration substances) The actually measured pHs of the mobile phases was 5.0, 8.2 and 9.7, but it is noted that a pH determination of an acetonitrile/water mixture may be inaccurate. Injection amounts: Calibration substances: 0.0005 – 4.74µg, 0.1µg test item, 0.01µg thiourea. pH 4 The calibration curve had a correlation coefficient r of 0.9775 (y = 2.7941x+2.9859). pH 7	

	The calibration curve had a correlation coefficient r of 0.9774 (y = 2.8029x+2.9973). pH 9 The calibration curve had a correlation coefficient r of 0.9775 (y = 2.8055x+2.9825). Calibrations and determinations were ran in duplicate. The data of the duplicates was not included in the summary, but produced comparable results to the first runs.
Results and discussion	The log Pow determined was 5.5. The results show no pH dependence. Although the study performed according to the guideline, it would have been preferable to have at least one additional reference at or near the log Pow of transfluthrin to reduce the error in the determination (only one reference had a higher log Pow than transfluthrin). In addition, the three reference substances at higher log Pow were all above the calibration curve, suggesting the log Pow of transfluthrin may be underestimated. This RMS considers this to be insufficient reason to reduce the reliability of the study as the guideline does not demand a minimum of more than one reference substance at a higher log Pow than the test substance. Lastly, the OECD guideline indicates the reference substances should preferably structurally related to the test substance. This is considered not feasible in the case of transfluthrin. Considering the test substance is not surface active and does not dissociate, the choice of the reference substances is accepted, taking into account the note above.
Conclusion	The log Pow of transfluthrin is 5.5 at pH 4, 7 and 9 at 25°C, determined using the HPLC method as described in OECD guideline 117.
Reliability	1
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	3 April 2018
Materials and Methods	Not applicable; summary by RMS
Results and discussion	Not applicable; summary by RMS
Conclusion	The log Pow of transfluthrin is 5.5, independent of pH
Reliability	1
Acceptability	Acceptable
Remarks	The study was provided in the context of the CLH procedure after substance approval following the evaluation according to the requirements of Directive 98/8/EC. The EU dossier did not include an experimentally determined log Pow of transfluthrin, but was based on model estimations considering a BCF was available (estimations were 5.94 using Bioloom and 6.17 based on EPIWIN v3.2). An experimentally determined log Pow value is preferred over that of a model estimation. The new data is therefore considered to replace these estimations.

2 TOXICOKINETICS (ABSORPTION, METABOLISM, DISTRIBUTION AND ELIMINATION)

2.1.1 Study 1 - Metabolism in female rats

Section A 6.2/02	**Metabolism Study in Animal**
BPD Annex Point IIA6.2	Metabolism in female rats

		Official use only
	Reference	
Data protection	Yes.	
Data owner	Bayer CropScience AG	
Companies with letter of access	None	
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.	
	Guidelines and Quality Assurance	
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	
GLP	Yes	
Deviations	None.	
	MATERIALS AND MethodS	
Test material		
Lot/Batch number	Sample-ID: KATH 6316	
	Batch No. SEL 1520	
Specification	As given in section 2	
Description	Solid, dried in vacuo	
Purity	Radiolabelled transfluthrin – radiochemical purity >99% (determined by HPLC and TLC)	
	Non-radiolabelled transfluthrin – chemical purity >99% (determined by HPLC)	
Stability	in a freezer at ≤ -18 °C until preparation of the stock solution	
Radiolabelling	Radiolabeled position: Methylene-^{14}C	
Specific activity	3.67 MBq/mg = 99.19 µCi/mg = 2.2 x 10^5 dpm/µg = 36.82 Ci/mol	
Test Animals	*Non-entry field*	
Species	Rat (*Rattus norvegicus domesticus*)	
Strain	Wistar Hsd/Cpb: WU	
Source	Harlan Nederland, Kreuzelweg 53, NL-5960 NM Horst, Netherlands	
Sex	Female	
Age/weight at study initiation	Approx. 8 – 9 weeks (female rats) at the time of delivery	
	195 – 218 g at fosing	
Number of animals per group	12	
Control animals	No	

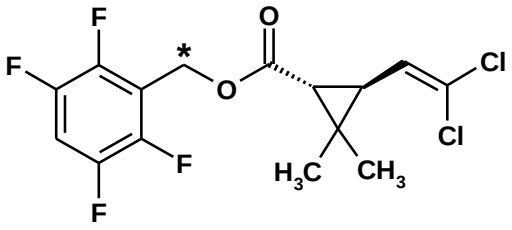
Administration/ Exposure	Oral
Duration of treatment	Single dosing
Frequency of exposure	Single dosing
Post exposure period	1, 5 and 24 hours
Type	gavage
Concentration	3 mg/kg bw. Details are given in Table A6.2/02-1
Vehicle	0.5% aqueous Cremophor EL
Concentration in vehicle	1.54 mg/mL Specific activity: 5.67 MBq/mL (3.4 x 10^8 dpm/mL)
Total volume applied	2 mL
Controls	Not applicable
Sample collection	The collection intervals for the respective samples are shown in Table A6.2/02-2
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
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
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.
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
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.
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.
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. 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.
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.
Identification / Characterisation and Quantitation of Residues	
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.
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.
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
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. Results and Discussion
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:
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.
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}).
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).
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. 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
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.
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
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
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
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
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
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.
	Applicant's Summary and conclusion
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:



✱ 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

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 [1 and 2]

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

[1] 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)

[2] 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)

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 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.
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.
Reliability	1
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	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 Anonymous, 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							2.86	212
2	10.8	18	1.0 mL/0.6 mg	3	133 495 112	0.61	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	1	3	Ratio	Decline by
Sacrifice [h post admin.]	1	24	1/24 h	[%]
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. Sacrifice [h p. dosing] Sampling period [h]		1 1 h 0 - 1 h		2 5 h 0 - 5 h		3 24 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. Sacrifice [h p. dosing]		1 1 h			2 5 h			3 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 unclea-ved 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. Sacrifice [h p. dosing]		1 1 h			2 5 h			3 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. / Sacrifice [h p. dosing]		1 1 h			2 5 h			3 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

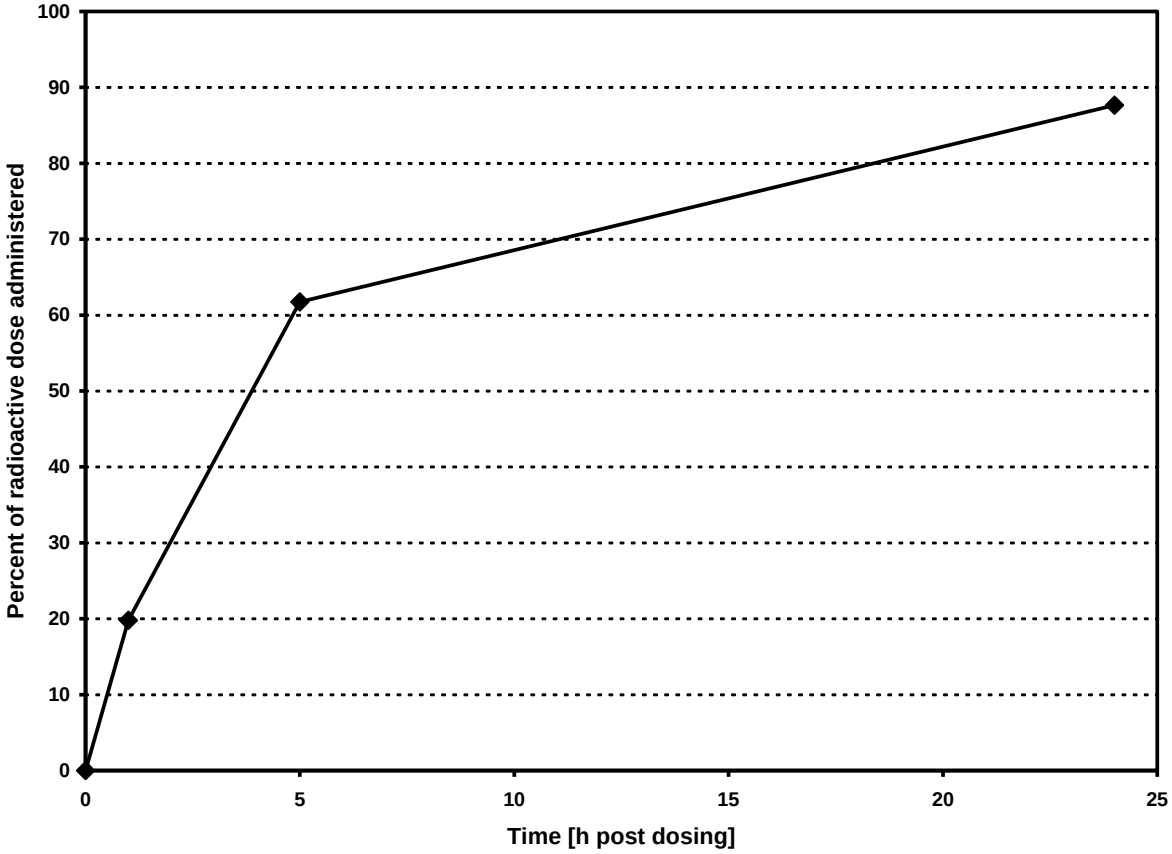


Figure A6.2/02-1: Tests 1 – 3: Time course of total radioactivity in urine

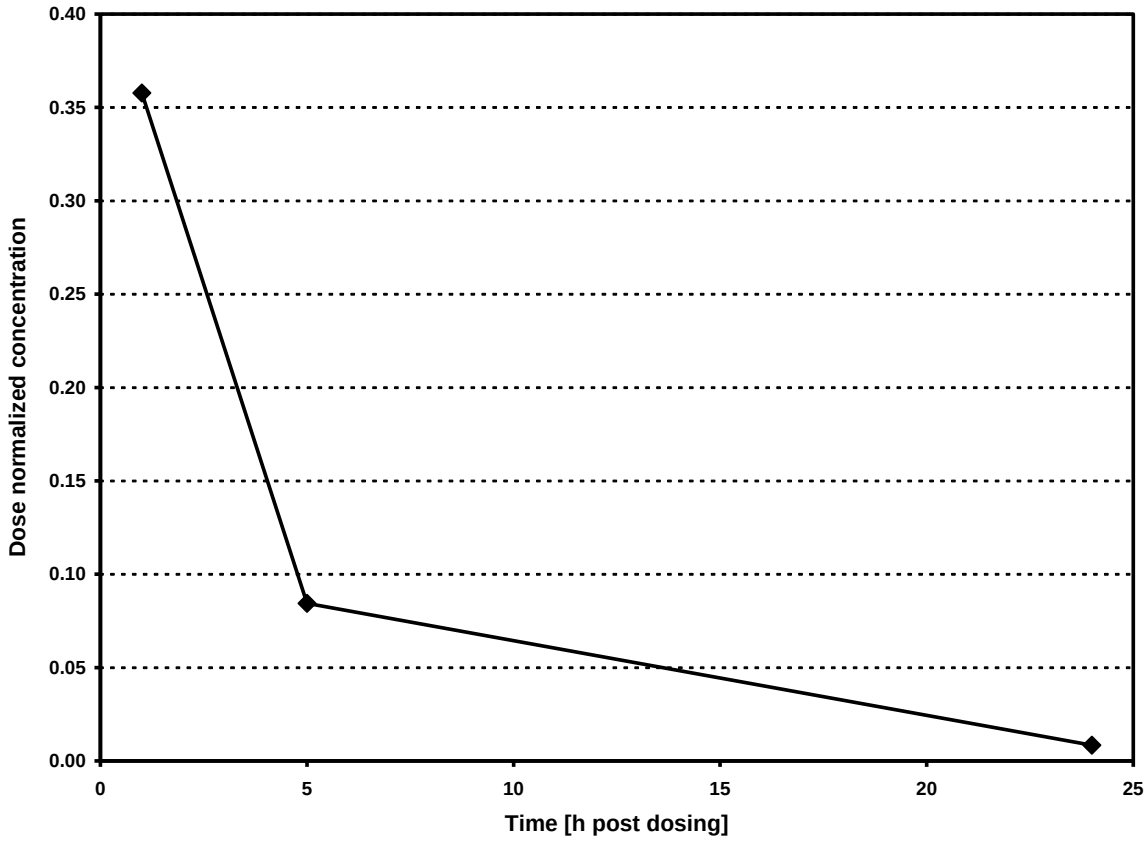


Figure A6.2/02-2: Tests 1 – 3: Time course of total radioactivity in blood

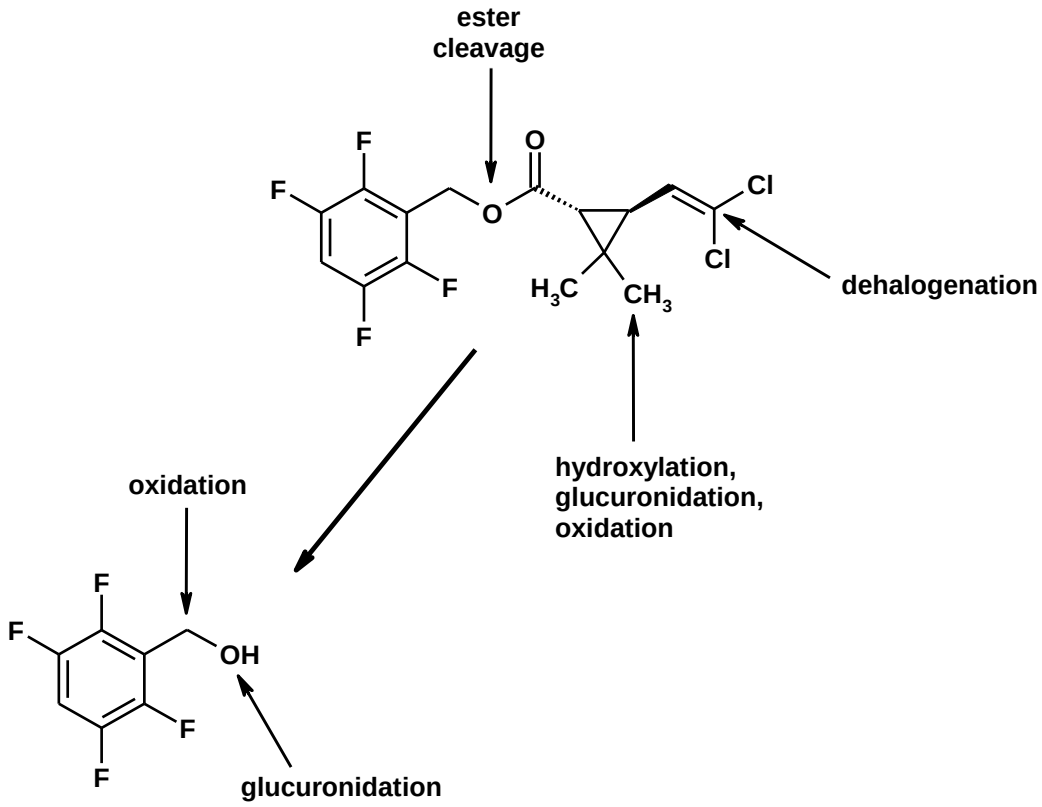
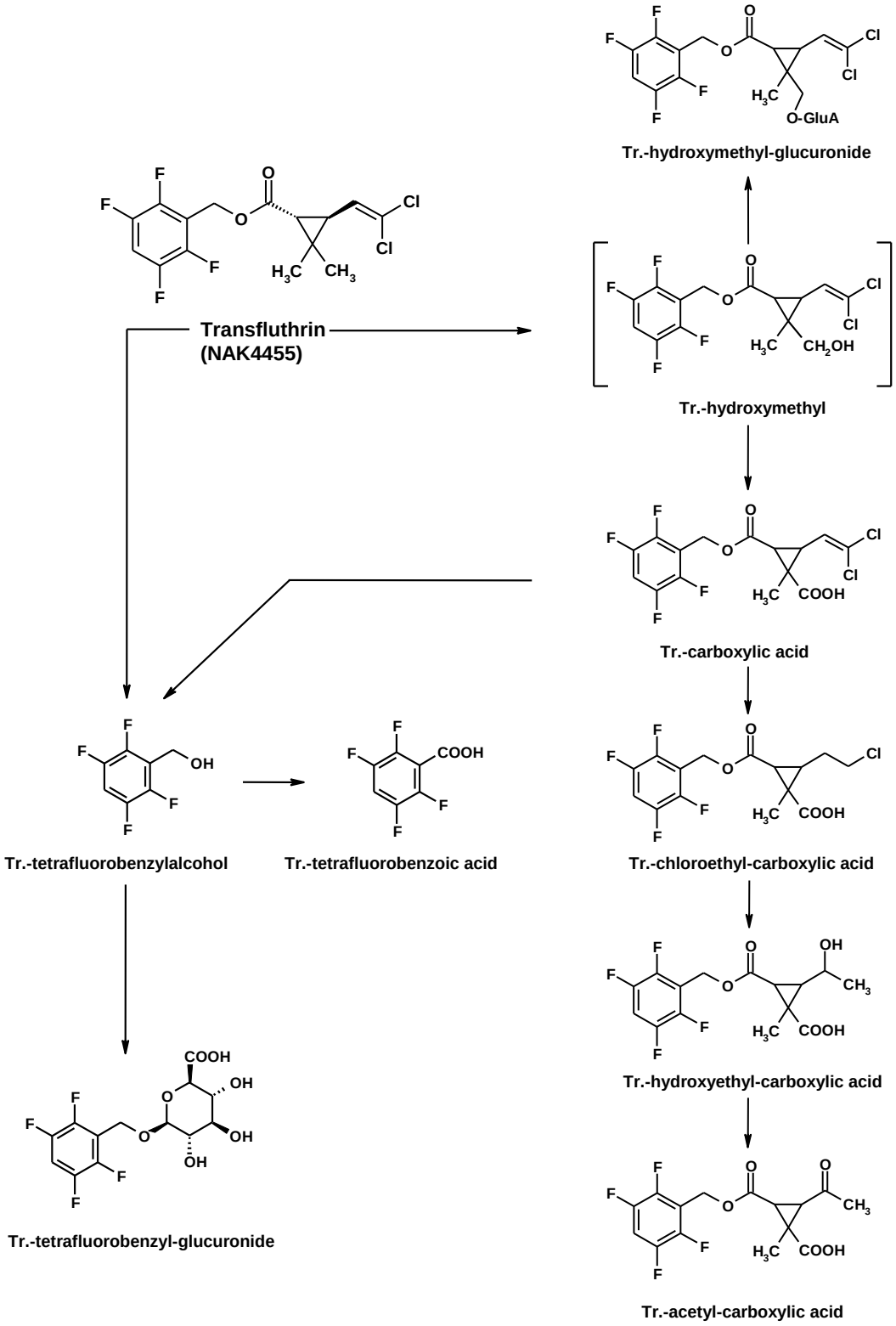


Figure A6.2/02-4: positions in the molecule, which are involved in the metabolic reactions



[Tr. = Transfluthrin]

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

3 HEALTH HAZARDS

Acute toxicity

3.1 Acute toxicity - oral route

3.1.1 Animal data

3.1.1.1 Study 1 - Basic Toxicokinetics in the Rat

Doc. IIIA Section 6.2 **BPD Data set IIA/ Annex Point IIA.6.2**	**Metabolism Studies in Animals – Basic Toxicokinetics in the Rat**	
	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience (Mobay Corporation)*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	No, but methods used are comparable to EC Method B.36.	
GLP	*Yes*	
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. MATERIALS AND MethodS	
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.*	
Lot/Batch number	*Radiolabelled benfluthrin – Lot no. 4813/1 from Bayer AG* *Non-radioactive benfluthrin – vial no 1008, Bayer reference no. 88032SELB01.*	
Specification	As given in section 2	
Description	*Physical appearance not described.*	
Purity	*Radiolabelled benfluthrin – radiochemical purity >99%* *Non-radiolabelled benfluthrin – 97.8% purity*	

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.*
Radiolabelling	*Methylene - ^{14}C benfluthrin*
Test Animals	
Species	Rat
Strain	*Wistar*
Source	*SASCO, Inc. Omaha, Nebraska, USA*
Sex	*Male and female*
Age/weight at study initiation	*Animals were received weighing from 140 to 200 g bw*

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*

Control animals	No
Administration/ Exposure	*Oral*
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.*
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*

Volume applied	The test compound was administered by gavage in approximately 0.5 ml, adjusted for a.s. content		
Vehicle	Polyethylene glycol		
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.		
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.	
	Cage rinse	Upon completion of each experiment, the walls of the cages were rinsed with methanol. The rinses were collected and radioassayed.	
	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.	
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
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.		

	Results and Discussion	
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
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.*	

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
Toxic effects, clinical signs	*No effects were described.*	
Dermal irritation	*No effects were described; this was an oral study.*	
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

	Applicant's Summary and conclusion	
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.*	
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.* *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.*	X
Conclusion		
Reliability	*1*	
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, <u>the tetrafluorobenzyl-methylene moiety of</u> 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 <u>containing the tetrafluorobenzyl-methylene moiety</u> 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	

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	M	45.4	39.7	2.9	87	8.6	2.0	8.7:1	1.6	1.2
(5 animals each gender)	F	38.1	48.3	2.7	86	9.5	2.2	7.8:1	1.5	0.9
HDE	M	41.5	44.8	4.6	87	7.7	2.8	8.7:1	1.8	1.0
(5 animals each gender)	F	47.2	40.9	2.5	90	6.6	1.4	11.2:1	1.1	1.0
SHDE	M	39.8	34.2	2.0	74	22.7	1.2	3.1:1	0.6	0.6
(2 animals each gender)	F	34.9	45.9	4.4	83	13.9	1.2	5.5:1	0.8	1.1
LDCE	M	39.5	47.4	2.1	88	8.3	2.0	8.8:1	1.3	0.6
(5 animals each gender)	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	M	62	25	<1	87
(5 animals each gender)	F	67	20	1	88
HDE	M	54	30	2	86

(5 animals each gender)	F	69	18	3	90
SHDE	M	59	14	<1	73
(2 animals each gender)	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.				

3.1.1.2 Study 2 - Acute oral toxicity in the mouse

Doc. IIIA/Section A6.1.1 **Acute Toxicity**
BPD Data set IIA/ Annex Point VI.6.1.1 ***Acute oral toxicity in the mouse***

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD 401 (1981)	
GLP	Yes	
Deviations	No MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	130187	
Specification	As given in sections 2 and 3	
Description	Dark brown solid/liquid	
Purity	94.5%	
Stability	Test substance was stored in a laboratory cabinet (at 21.5-24°C) and kept stable throughout the study.	
Test Animals		
Species	Mouse	
Strain	NMRI (SPF-Han)	
Source	Versuchstierzucht Winkelmann, Borchen, Kreis Paderborn	
Sex	Male and female	
Age/weight at study initiation	4 – 5 weeks Weight range of 20 – 28 g (males) and 20 – 25 g (females)	
Number of animals per group	5 mice/sex/group	
Control animals	No	
Administration/ Exposure	Oral	
Postexposure period	14 days	
	Oral	
Type	Gavage to fasted animals (16 hours)	
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	

Vehicle	Polyethylene glycol E 400	
Concentration in vehicle	Not stated	
Total volume applied	5 or 10 mL/kg	
Controls	None	
Examinations	Clinical observations, necropsy, body weight	
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	
Further remarks	None	
	Results and Discussion	
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
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.	
Other	No other significant effects	
LD_{50}	LD_{50} (male): 583 mg/kg bw LD_{50} (female): 688 mg/kg bw	
	Applicant's Summary and conclusion	
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.	
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.
Conclusion	
Reliability	1
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	*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}.

3.1.2 Human data

No data available.

3.1.3 Other data

No data available.

3.2 Acute toxicity - dermal route

3.2.1 Animal data

Doc. IIIA/Section A6.1.2 **Acute Toxicity**
BPD Data set IIA/ Annex Point VI.6.1.2 ***Acute dermal toxicity in the mouse***

		Reference	**Official use only**
Data protection		Yes	
Data owner		Bayer CropScience	
Companies with letters of access			
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	
		Guidelines and Quality Assurance	
Guideline study		Yes	
		OECD 402 (1987)	
		US EPA OPPTS § 870.1200 (1998)	
		Directive 67/548/EEC Annex V, B.3 (1992)	
GLP		Yes	
Deviations		No	
		MATERIALS AND MethodS	
Test material		NAK 4455 (transfluthrin)	
Lot/Batch number		816779504	
Specification		As given in sections 2 and 3	
Description		Clear, colourless liquid	
Purity		95.8%	
Stability		Test substance was stored at room temperature and kept stable throughout the study.	
Test Animals			
Species		Mouse	
Strain		NMRI:WU	
Source		Harlan Winkelmann GmbH, Borchen, District of Paderborn	
Sex		Male and female	
Age/weight at study initiation		8 weeks	
		Weight range of 36 – 41 g (males) and 25 – 30 g (females)	
Number of animals per group		5 mice/sex/group	
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	
Examinations		Clinical observations, necropsy, body weight	
Method of determination of LD_{50}		No statistical analysis was necessary for determining the LD_{50}.	
Further remarks		None	
		Results and Discussion	
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.	
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.	
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.	
LD_{50}		The LD_{50} was > 4000 mg/kg bw (highest dose tested) for both males and females.	
		Applicant's Summary and conclusion	
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.	
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.
Conclusion	
Reliability	1
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	*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	

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		

3.2.2 Human data

No data available.

3.2.3 Other data

No data available.

3.3 Acute toxicity - inhalation route

3.3.1 Animal data

Doc. IIIA/Section A6.1.3 Acute Toxicity
BPD Data set IIA/ Annex Point VI.6.1.3 ***Acute inhalation toxicity in the rat***

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD 403 (1981)	
	EC B.2 (1984)	
	FIFRA § 81-3 (1984)	
GLP	Yes	
Deviations	No	

	MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
Lot/Batch number	250 987 (mixed batch)
Specification	As given in sections 2 and 3
Description	Crystalline mass, brownish, solid
Purity	94.5%
Stability	Test substance was stored in at room temperature with light excluded; stability was ensured for period of study
Test Animals	
Species	Rat
Strain	Bor: WISW (SPF-Cpb)
Source	Versuchstierzucht Winkelmann, Borchen, Kreis Paderborn
Sex	Male and female
Age/weight at study initiation	2 – 3 months Weight range of 160 – 200 g
Number of animals per group	5 mice/sex/group
Control animals	Yes, but not concurrent
Administration/ Exposure	Inhalation
Postexposure period	14 days
	Inhalation
Concentrations	Nominal concentration 5000 [mg/m³] Analytical concentration 513 [mg/m³]
Particle size	MMAD (mass median aerodynamic diameter) 1.44 [µm] ± GSD (geometric standard deviation) 1.42 [µm]
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.
Type of exposure	Nose/head only
Vehicle	Polyethylene glycol E 400:ethanol (1:1)
Concentration in vehicle	25% dilution (maximum achievable concentration)
Duration of exposure	4 h
Controls	Vehicle exposure (not concurrent)
Examinations	Clinical observations, necropsy, body weight, reflexes
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
Further remarks	None
	Results and Discussion
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.
Pathology	No abnormal findings were seen in animals sacrificed at end of study.
Other	No other significant effects
LD_{50}	LD_{50} (male): > 513 mg/m^3 LD_{50} (female): > 513 mg/m^3

	Applicant's Summary and conclusion
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 □m, the geometric standard deviation was approximately 1.42 □m, 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.
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.
Conclusion	
Reliability	1
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	*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***	***Time of death (range)***	***Observations***

	investigated		
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}.

3.3.2 Human data

No data available.

3.3.3 Other data

No data available.

3.4 Skin corrosion/irritation

3.4.1 Animal data

Doc. IIIA/Section A6.1.4/01 Acute Dermal Irritation
BPD Data set IIA/ Annex Point VI.6.1.4 Skin irritation study in the rabbit

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD 404 (1981)	
	EC B.4. Acute toxicity (skin irritation) 1984	
	FIFRA Guideline No. 81.5 (1984) Primary dermal irritation	
GLP	Yes	
Deviations	No	
	MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	

Doc. IIIA/Section A6.1.4/01 **BPD Data set IIA/ Annex Point VI.6.1.4**	Acute Dermal Irritation Skin irritation study in the rabbit
Lot/Batch number	NAK 4455-III-4733 of June 5, 1986
Specification	As given in sections 2 and 3
Description	Colourless liquid
Purity	95.0%
Stability	Guaranteed stability to December 26, 1986
Test Animals	
Species	Rabbit
Strain	HC:NZW
Source	Interfauna UK Ltd.
Sex	Female
Age/weight at study initiation	Adult Weight range of 3.0 – 3.7 kg
Number of animals per group	Three
Control animals	No
Administration/ Exposure	Dermal
Application	
Preparation of test substance	Test substance was used as delivered.
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.
Occlusion	Semiocclusive
Vehicle	None
Concentration in vehicle	Not applicable, no vehicle used
Total volume applied	0.5 ml per animal
Removal of test substance	Water
Duration of exposure	4 h
Postexposure period	1 week
Controls	Opposite flank was treated similarly, but water was used instead of test material.
Examinations	
Clinical signs	No
Dermal examination	Yes
Scoring system	Draize
Examination time points	60 min, 24 h, 48 h, 72 h and 1 week
Other examinations	None
Further remarks	
	Results and Discussion
Average score	
Erythema	Average score for all animals at 24, 48, 72 h was 0.
Oedema	Average score for all animals at 24, 48, 72 h was 0.
Reversibility	Not applicable
Other examinations	None stated
Overall result	Primary irritation score 0.0 (total score of skin reaction over 72 hours)
	Applicant's Summary and conclusion
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

Doc. IIIA/Section A6.1.4/01 BPD Data set IIA/ Annex Point VI.6.1.4	Acute Dermal Irritation Skin irritation study in the rabbit
	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.
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.
Conclusion	
Reliability	1
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	*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	

3.4.2 Human data

No data available.

3.4.3 Other data

No data available.

3.5 Serious eye damage/eye irritation

3.5.1 Animal data

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	
	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD 405 (1981)	
	EC B.5. Acute toxicity (eye irritation) 1984	
	FIFRA Guideline No. 81.4 (1984) Primary eye irritation	
GLP	Yes	
Deviations	No	
	MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	NAK 4455-III-4733 of June 5, 1986	
Specification	As given in sections 2 and 3	
Description	Colourless liquid	
Purity	95.0%	
Stability	Guaranteed stability to December 26, 1986	
Test Animals		
Species	Rabbit	
Strain	HC:NZW	
Source	Interfauna UK Ltd.	
Sex	Female	
Age/weight at study initiation	Adult Weight range of 3.0 – 3.7 kg	
Number of animals per group	Three	
Control animals	No	
Administration/ Exposure		
Preparation of test substance	Test substance was used as delivered	
Amount of active substance instilled	0.1 ml	
Exposure period	24h (Single instillation, eye was rinsed with physiological saline after exposure period)	
Postexposure period	1 week	
Examinations		

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	
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.	
Scoring system	Draize	
Examination time points	60min, 24h, 48h, 72h and 1 week	
Other investigations	None	
Further remarks	None	
	Results and Discussion	
Clinical signs	*No abnormality was observed in any animals during the experimental period.*	
Average score		
Cornea	Average scores for all animals at 24, 48, 72 h was 0	
Iris	Average scores for all animals at 24, 48, 72 h was 0	
Conjunctiva		
Redness	Average scores for all animals at 24, 48, 72 h was 0	
Chemosis	Average scores for all animals at 24, 48, 72 h were 0.3, 0, 0	
Reversibility	Yes Chemosis was completely reversible within 24 hr	
Other	None	
Overall result	*The maximum mean total score (MMTS) was 0, 72 hrs after application.*	
	Applicant's Summary and conclusion	
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.	
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
Conclusion		X
Reliability	1	
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	*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	

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

3.5.2 Human data

No data available.

3.5.3 Other data

No data available.

3.6 Respiratory sensitisation

3.6.1 Animal data

No data available.

3.6.2 Human data

No data available.

3.6.3 Other data

No data available.

3.7 Skin sensitisation

3.7.1 Animal data

Doc. IIIA/ Section A6.1.5	**Skin sensitisation**
BPD Data set IIA/ Annex Point VI.6.1.5	Skin sensitisation in the Guinea pig (Buehler Test)

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD 406 (1981) FIFRA Guideline No. 81.6 (1984) EC Guideline 84/449 Acute toxicity—Sensitisation of skin (1984)	
GLP	Yes	
Deviations	No MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	250987	
Specification	As given in sections 2 and 3	
Description	Solid brown mass (at room temp), after melting (at 50°C): brown-yellow clear liquid	
Purity	95.0%	
Stability	Stable until October 27 1988	
Preparation of test substance for application	for induction: used as delivered (melted) for challenge: used as delivered (melted)	
Pretest performed on irritant effects	No	
Test Animals		
Species	Guinea pigs	
Strain	DHPW (SPF)	
Source	Winkelmann, Borchen, Kreis Paderborn	

Doc. IIIA/ Section A6.1.5	**Skin sensitisation**
BPD Data set IIA/ Annex Point VI.6.1.5	Skin sensitisation in the Guinea pig (Buehler Test)
Sex	Male
Age/weight at study initiation	5 – 7 weeks Body weight range of 348 to 428 g.
Number of animals per group	12 animals per group
Control animals	Yes, 12 for first challenge, 12 kept ready for possible second challenge
Administration/ Exposure	Non-Adjuvant
Induction schedule	Day 0 – day –7 – day 14
Way of Induction	Topical Occlusive
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.
Concentration Freunds Complete Adjuvant (FCA)	Not applicable
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.
Concentrations used for challenge	0.5 ml undiluted test compound
Rechallenge	No
Scoring schedule	24h, 48h, 72h after challenge
Removal of the test substance	Test substance was removed after 24 hours; any test material residue was removed with sterile physiological saline solution.
Positive control substance	Not stated, however the strain's sensitivity for sensitisation tests is checked at regular intervals and documented.
Examinations	
Pilot study	No
Further remarks	
	Results and Discussion
Results of pilot studies	Not applicable
Results of test	
24h after challenge	No skin reactions were observed in treated (0/12) or control (0/12) animals.
48h after challenge	No skin reactions were observed in treated (0/12) or control (0/12) animals.
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.
Overall result	Based on the results obtained NAK 4455 was considered to have no sensitising potential under the conditions of this test.
	Applicant's Summary and conclusion
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.
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.
Conclusion	
Reliability	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 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	

Doc. IIIA/ Section A6.1.5 **Skin sensitisation**
BPD Data set IIA/ Annex Point VI.6.1.5 Skin sensitisation in the Guinea pig (Buehler Test)

	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 A6.1.5-1. 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

3.7.2 Human data

No data available.

3.7.3 Other data

No data available.

3.8 Germ cell mutagenicity

3.8.1 In vitro data

3.8.1.1 Study 1 - In-vitro gene mutation study in bacteria

Doc. IIIA/ Section A6.6.1	Genotoxicity in vitro
BPD Data set IIA/ Annex Point VI.6.1	***In-vitro* gene mutation study in bacteria (*Salmonella* and *E.coli* reverse mutation assay)**

			Official use only
		Reference	
Data protection		Yes	
Data owner		*Bayer CropScience*	
Companies with letters of access			
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)*	
GLP		*Yes*	
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	*S. typhimurium :* 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.	
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.*
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.*
		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.

3.8.1.2 Study 2 - In-vitro cytogenetic assay in mammalian cells

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

Doc. IIIA/ 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*)	
	Reference	**Official use only**
Data protection	Yes	
Data owner	*Bayer AG*	
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	
	Guidelines and Quality Assurance	
2.1 Guideline study	*Yes*	
	The methods used are comparable to OECD 473 (July 1997)	
GLP	*Yes*	
Deviations	No	X
	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	
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.	
Deficiencies / Proficiencies	Not applicable	
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).	

Doc. IIIA/ 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*)	
Positive control	*The following substances were used:* *Mitomycin C (MMC)* *Cyclophosphamide (CP)*	
Administration / Exposure; Application of test substance		
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.*	
Way of application	*Solutions of transfluthrin in dimethylsulphoxide (DMSO) were applied directly to cells within the culture medium.*	
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.* *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.*	X X X
Other modifications		
Examinations		

Doc. IIIA/ 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*)	
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.*	
	Results and Discussion	
4.1 Genotoxicity		
4.1.1 without metabolic activation	*No*	
4.1.2 with metabolic activation	*No*	
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.	
	Applicant's Summary and conclusion	

Doc. IIIA/ 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*)	
.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.	
		The test material was dissolved in DMSO, and stability was confirmed. Human lymphocyte primary cell cultures were incubated for 48 hours at 37°C 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.	X
		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.	
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.	
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.*	
Reliability		*1*	
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	*- 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 Pallen) and all test data are clearly within the historical control range of the performing laboratory (HCD and contained in position paper by Wason, March 2011). More detailed argumentation is presented in position paper (Wason, 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	

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	Evaluated metaphases	400	400	400	300	400

endoreduplicated cells	% 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.					

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.					

3.8.1.3 Study 3 - In vitro Micronucleus Study in Human peripheral Blood Lymphocytes

Document IIIA/ Section 6.6.2/02 **Annex Point IIA VI.6.6**	**Genotoxicity in vitro** *In vitro* Micronucleus Study in Human peripheral Blood Lymphocytes

	Reference	Official use only
Data protection	Yes	
Data owner	Bayer CropScience AG	

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 Guidelines and Quality Assurance
Guideline study	based on OECD guideline 487 (2010)
GLP	Yes
Deviations	None that would prejudice the validity of the study in anyway MATERIALS AND MethodS
Test material	Transfluthrin
Lot/Batch number	Batch No. PNLO000030
Specification	As given in sections 2
Description	Colourless melt
Purity	97.6%. 1-R trans isomer: 99.1% area 1 S- trans isomer: 0.9% area
Stability	stored at 15-25°C protected from light
Study Type	Micronucleus test in vitro
Organism/cell type	Human Blood Lymphocytes. Blood from two healthy, non-smoking female volunteers from a panel of donors at Covance
Deficiencies / Proficiencies	Not applicable
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).
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
Administration / Exposure; Application of test substance	
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**
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 **Error! Reference source not found.**. 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
Pre-incubation time	Not applicable
Other modifications	Not applicable
Examinations	
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.
	Results and Discussion
Genotoxicity	
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**
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. See **Table A6.6.2/02-4**
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
	Applicant's Summary and conclusion
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.
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**
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
Reliability	1
Deficiencies	None reported

	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: Transfluthrin, 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: Transfluthrin, 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: Transfluthrin, 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
[#]95[th] percentile of the observed range
NS = Not significant
ND = Not determined

3.8.1.4 Study 4 - In-vitro gene mutation assay in mammalian cells

Doc. IIIA/ Section A6.6.3	**Genotoxicity in vitro**
BPD Data set IIA/ Annex Point VI.6.3	In-vitro gene mutation assay in mammalian cells

			Official use only
		Reference	
Data protection		Yes	
Data owner		Bayer AG	
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	
		Guidelines and Quality Assurance	
2.1	Guideline study	Yes	
		The methods used are comparable to OECD 476 (1997)	
GLP		Yes	
Deviations		No	
		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.
Organism/cell type	Chinese hamster ovary (CHO) cell line, subclone CHO-K1-BH_4
Deficiencies / Proficiencies	Not applicable
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.
Positive control	The following substances were used: Ethylmethanesulfonate (EMS), 0.9 mg/ml in non-activation trials Dimethylbenzanthracene (DMBA), 20 □g/ml in S9 activation trials
Administration / Exposure; Application of test substance	
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.
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.
Pre-incubation time	None

Doc. IIIA/ Section A6.6.3	**Genotoxicity in vitro**
BPD Data set IIA/ Annex Point VI.6.3	In-vitro gene mutation assay in mammalian cells
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.
Other modifications	None
Examinations	
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.
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 25×10^{-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.
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.
Confirmatory Assay?	Yes; second trial for confirmation of results of first trial
	Results and Discussion

Doc. IIIA/ Section A6.6.3	**Genotoxicity in vitro**	
BPD Data set IIA/ Annex Point VI.6.3	In-vitro gene mutation assay in mammalian cells	
4.1	Genotoxicity	
4.1.1	without metabolic activation	No
4.1.2	with metabolic activation	No
Cytotoxicity		Cytotoxicity was noted at and above 50 □g/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 □g/ml.
		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.
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.
Conclusion		From these results transfluthrin is considered nonmutagenic in the CHO Forward Mutation Assay, both with and without metabolic activation.
Reliability		1
Deficiencies		No

	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	

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 □g/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 □g/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 □g/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					

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 □g/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 □g/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 □g/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 □g/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 □g/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 □g/ml DMBA * = p<0.05 *= p<0.05; not considered biologically significant because of the lack of a dose-response					

3.8.2 Animal data

3.8.2.1 Study 1 - In vivo mutagenicity study, micronucleus test in mice

Doc. IIIA/ Section A6.6.4/02	**Genotoxicity in vivo**
BPD Data set IIA / Annex Point VI.6.4	In vivo mutagenicity study, micronucleus test in mice

	Reference	Official use only
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
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	
GLP	Yes	
Deviations	Yes; however none of these deviations were considered to affect the validity of the study. MATERIALS AND MethodS	
Test material	Transfluthrin (NAK 4455)	
Lot/Batch number	PNLO000031	
Specification	As given in section 2	
Description	Colourless melt	
Purity	98.4% pure from analysis of 2011-07-25	
Stability	Storage conditions 10 to 30°C. Stable for at least 2 years if stored as recommended.	
Test Animals		
Species	mouse	
Strain	NMRI	
Source	Charles River Laboratories, 97633 Sulzfeld, Germany	
Sex	Male and female	
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)	
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	
Control animals	Yes	
Administration/ Exposure	Oral	
Number of applications	1	
Interval between applications	Not applicable	
Post exposure period	24 h and/or 48 h after treatment Oral	

Type	Gavage
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.
Vehicle	Polyethylene glycol 400 (PEG 400)
Concentration in vehicle	Not stated. As calculated from the study: 375 mg/kg in 5 mL/kg = 75 mg/mL
Total volume applied	10 ml/kg b.w.
Controls	yes
Substance used as Positive Control	Cyclophophamide (CPA) dose was 40 mg/kg bw administered in sterile water.
Positive control	PEG 400 only.
Experimental Procedures	This study was conducted in accordance with OECD guideline 474.
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
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
Examinations	
Clinical signs	General symptoms up to 48 hours, including abdominal position, ruffled fur, tremors, eyelid closure, excitement, Straub's phenomenon and death were monitored
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
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.
	Results and Discussion.
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.
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 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
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
Other	None
	Applicant's Summary and conclusion
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 (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..
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
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
Reliability	1
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 b.w.**	**sampling time (h)**	**PCEs with micronuclei (%) ± sd**	**Range (*)**	**PCE per 2000 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

3.8.3 Human data

No data available.

3.8.4 Other data

No data available.

3.9 Carcinogenicity

3.9.1 Animal data

3.9.1.1 Study 1 - 2-year oral rat study

Doc IIIA/Section A 6.7/01	**Chronic Toxicity / Carcinogenicity**
BPD Data set IIA/ Annex Point VI. 6.5/6.7	2-year oral rat study

		Official use only
	Reference	
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD 453 (1981)	
	US EPA FIFRA § 83-5 (1984)	
GLP	Yes	
Deviations	No	
	MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	Mixed batch no: 130187, from 10.11.87: 250987	
Specification	As given in sections 2 and 3	
Description	Brown-yellow clear liquid after heating to 50ºC	
Purity	95.0% (130187), 94.5% (250987)	
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.	
Test Animals		
Species	Rat	
Strain	Wistar; Bor:WISW (SPF-Cpb)	
Source	Winkelmann, Borchen	
Sex	Male and female	
Age/weight at study initiation	4-6 weeks Weight range at start of study 54-78 g (males) and 52-73 g (females)	
Number of animals per group	70 rats/sex/group	
at interim sacrifice	10 animals/group/sex at 12 months	
at terminal sacrifice	60 animals/group/sex	

Control animals	Yes
Administration/ Exposure	Oral (dietary)
Duration of treatment	25 months
Interim sacrifice(s)	After 12 months
Final sacrifice	After 25 months
Frequency of exposure	Daily (continuous in diet)
Postexposure period	None
	Oral
Type	In food
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
Vehicle	Moistened with peanut oil/ mixed into food (1% final concentration)
Concentration in vehicle	N/A
Total volume applied	Not applicable
Controls	Diet with peanut oil
Examinations	
Body weight	Yes, before administration of first dose and then weekly.
Food consumption	Yes, measured weekly.
Water consumption	Yes, measured weekly.
Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
Macroscopic investigations	Location and progression of palpable masses, skin tumours were recorded
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.
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:
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	
Urinalysis	Yes	
	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	
Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at interim and final sacrifice	
Organ Weights	Yes	
	From:	All animals at interim or final sacrifice
	Organs:	Liver, kidneys, adrenals, testes, spleen, brain, heart, and lungs
		Other
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, urethra, urinary bladder, uterus, vagina and any other tissue showing changes.
		Other
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.
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.
Further remarks	
	Results and Discussion
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.
Food consumption	No treatment related effects were seen.
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.
Clinical signs	No treatment related effects were seen
Macroscopic investigations	No treatment related effects were seen.
Ophthalmoscopic examination	No treatment related effects were seen
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.
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 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.
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.
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.	
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.	
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.	
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.	
Time to tumours	No treatment related effects were seen.	
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	X

	frequency was above historical control data. Applicant's Summary and conclusion
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).
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. 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).
Conclusion	
Reliability	1
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*	
	bones F	0.548		0.894		0.637		0.848		1.249*		1.485*		2.949*		2.793*	
	teeth M	0.108		0.133		0.121		0.120		0.242*		0.290*		0.647*		0.677*	
	teeth 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

	Control data								**dose-response**	
	study		**low dose**		**medium dose**		**high dose**		**+ /**	
Parameter	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.

3.9.1.2 Study 2 - 2-year oral mouse study

Doc IIIA/Section A 6.7/02	**Chronic Toxicity/Carcinogenicity**
BPD Data set IIA/ Annex Point VI. 6.5/6.7	2-year oral mouse study

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD 451 (1981) US EPA FIFRA § 82-2 (1984)	
GLP	Yes	
Deviations	No MATERIALS AND MethodS	

Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	Mixed batch no: 250987	
Specification	As given in sections 2 and 3	
Description	Dark brown	
Purity	94.5- 95%	X
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.	
Test Animals		
Species	Mice	
Strain	B6C3F1	
Source	Charles River Wiga GmbH	
Sex	Male and female	
Age/weight at study initiation	5-6 weeks Weight range at start of study 18-24 g (males) and 15-20 g (females)	
Number of animals per group	60 mice/sex/group (+ extra 10 mice/sex/group for 0 and 1000 ppm groups)	
at interim sacrifice	10 animals/sex/group at 12 months	
at terminal sacrifice	50 animals/sex/group	
Control animals	Yes	
Administration/ Exposure	Oral	
Duration of treatment	24 months	
Interim sacrifice(s)	After 12 months	
Final sacrifice	After 24 months	
Frequency of exposure	Daily	
Postexposure period	None	
	Oral	
Type	In food	
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	
Vehicle	Moistened with peanut oil/ mixed into food	
Concentration in vehicle	Not applicable	
Total volume applied	Not applicable	
Controls	Diet with peanut oil	
Examinations		
Body weight	Yes, before administration of first dose and then weekly.	
Food consumption	Yes, measured weekly (based on extra 10 animals).	
Water consumption	Yes, measured weekly.	
Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.	

Macroscopic investigations	Palpable masses, skin tumours		
Ophthalmoscopic examination	No.		
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:	
Clinical Chemistry	Yes		
	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		
Urinalysis	No		
Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at 3-month, interim and final sacrifice		
Organ Weights	Yes		
	From:	All animals at 3-month, interim or final sacrifice	
	Organs:	Liver, kidneys, testes, spleen, brain, heart, ovaries, and lungs	
		Other	
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
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.
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.
Further remarks	
	Results and Discussion
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..
Food consumption	No treatment related effects were seen.
Water consumption	No treatment related effects were seen.
Clinical signs	No treatment related effects were seen,
Macroscopic investigations	No treatment related effects were seen.
Ophthalmoscopic examination	Not applicable
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).
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, 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.	
Urinalysis	Not applicable.	
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.	
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.	
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
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.	
Time to tumours	Not applicable	
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. Applicant's Summary and conclusion	
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
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
Conclusion		
Reliability	1	
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	*Urinalyses was not performed.* *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**	
Chol ester ol, 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 A6.7(02) -2. Results from carcinogenicity study

	Control data study		**low dose**		**medium dose**		**high dose**		**dose-response + /**	
Parameter	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)	-	-	-	-	**↑***	**↑**	**↑****	**↑****	+	+
Relative weight (interim 12 month)	-	-	-	-	-	**↓****	**↑****	-	-	-
Relative weight (final 24 month)	-	-	-	-	-	-	**↑****	**↑****	-	-

*p <0.05, ** p<0.01, *** P<0.001, - Not significantly different than control.

Right data

	Control data study		**low dose**		**medium dose**		**high dose**		**dose-response** + /	
Parameter	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**	**-**	**-**

3.9.2 Human data

No data available.

3.9.3 *In vitro* data (e.g. in vitro germ cell and somatic cell mutagenicity studies, cell transformation assays, gap junction intercellular communication tests)

No data available.

3.9.4 Other data (e.g. studies on mechanism of action)

3.9.4.1 Study 1

Section IIIA6.10/01 **Mechanism**
BPD Data set IIA/ Annex Point VI.7 *Clarification for bladder tumours, section 6.7*

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	

Companies with letters of access	
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
	Guidelines and Quality Assurance
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.
GLP	Yes
Deviations	Not applicable
	MATERIALS AND MethodS
Test material	As given in sections 2 and 3
Lot/Batch number	Batch no. 8169 79301
Specification	As given in sections 2 and 3
Description	Yellow to brown, liquid to solidified melt
Purity	95.8%
Stability	The active ingredient was stable in the feed mixture over a period of 10 days.
Test Animals	
Species	Rat
Strain	Wistar HsdCpb:wu (historically used at Bayer, so good data on spontaneous alterations)
Source	Harlan/Winkelmann, D33178 Borchen, Germany
Sex	female
Age/weight at study initiation	13-14 weeks, mean weight 218.5g (206-243g)
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
at interim sacrifice	At one week – 10 positive controls, 20 negative controls, 20 treated animals
at terminal sacrifice	At 4 weeks – 10 positive controls, 30 negative controls, 30 treated animals
Control animals	Yes
Administration/ Exposure	
Duration of treatment	1 or 4 weeks
Interim sacrifice(s)	After 1 week
Final sacrifice	After 4 weeks
Freqency of exposure	Daily
Postexposure period	None
	Oral
Type	In food
Concentration	5000 ppm, food, 327 mg/kg bw/day food consumption ad libitum
Vehicle	Dissolved in peanut oil before being mixed into food. Final: 5000 ppm NAK 4455 in Altromin 1321 diet with 1% peanut oil.
Controls	Negative control - Altromin 1321 diet with 1% peanut oil Positive control – 100 ppm sodium cacodylic acid (NaC) in Altromin diet

Section IIIA6.10/01	**Mechanism**
BPD Data set IIA/ Annex Point VI.7	*Clarification for bladder tumours, section 6.7*
Examinations	
Body weight	Yes
Food consumption	Yes
Water consumption	Yes
Clinical signs	Yes
Clinical Chemistry	No
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)
Pathology	Yes
Organ Weights	Liver and both kidneys
Histopathology	Urinary bladder only was examined.
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.
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.
Further remarks	
	Results and Discussion
Body weight	No effect
Food consumption	No effect
Water consumption	No effect
Clinical signs	Two animals in the treatment group showed increased urine excretion regarded as test substance related; however, mean water consumption was not different.
Macroscopic investigations	No effect
Urinalysis	Calcium imbalance noted in second week, and increased protein excretion at 5 weeks indicated treatment-related effects on the urinary system.
Organ Weights	Kidney weights were significantly increased after 4 weeks of treatment. Liver weights were significantly increased after 1 and 4 weeks of treatment.
Histopathology	Focal hyperplasia of the transitional epithelium of the urinary bladder was found in one of the 30 rats treated for 4 weeks.
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.

Section IIIA6.10/01 BPD Data set IIA/ Annex Point VI.7	**Mechanism** *Clarification for bladder tumours, section 6.7*
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.
Other	No effect on survival was noted. Applicant's Summary and conclusion
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.
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.
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.
Reliability	1
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*

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

3.9.4.2 Study 2 – clarification for bladder tumours

Doc. IIIA/ Section A6.10/02	Mechanism
BPD Data set IIA/ Annex Point VI.1.7	*Clarification for bladder tumours, section 6.7*

			Official use only
		Reference	
Data protection		Yes	
Data owner		*Bayer AG*	
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	
		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.*	
GLP		*No*	
Deviations		Not applicable	
		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**	
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	
Organism/cell type		Rat bladder epithelial explant cultures	
Deficiencies / Proficiencies		Not applicable	

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)[3], 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.
Positive control	*Mitomycine (purchased from Serva, Cat. No. 29805)*
Administration / Exposure; Application of test substance	

[3] 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.

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 °C 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 -80 °C until ready to be measured. Plates were thawed to RT, 100µl distilled water added and plates incubated at 37 °C for 1h. Plates were again placed at -80 °C 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%.*
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.

4.1 Cytotoxicity

Results and Discussion

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.

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.*
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.*
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
Reliability		***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	

3.9.4.3 Study 3 – 13 week oral toxicity in rats and mice

Section A 6.10/03	Mechanistic Study
BPD Annex Point IIIA6.7	13 week oral toxicity in rats and mice

	Reference	Official use only
Data protection	Yes.	
Data owner	Bayer CropScience AG	
Companies with letter of access	None	
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. Guidelines and Quality Assurance	
Guideline study	No, mechanistic study	
GLP	Yes	
Deviations	None MATERIALS AND MethodS	
Test material	Transfluthrin	
Lot/Batch number	ABIDTBN019	
Specification	As given in section 2	
Description	White melt	
Purity	99.6% w/w	
Stability	As given in section 2	
Test Animals		
Species	Rat and Mouse	
Strain	Wistar Hanover IGS [CRL: WI (Han)] rats (nulliparous and nonpregnant) B6C3F1 mice rats (nulliparous and nonpregnant)	
Source	Charles River Laboratories, Raleigh, NC, U.S.A.	
Sex	Female	
Age/weight at study initiation	Rats 8 weeks/ 150-193 g (start of treatment) Mice 8 weeks/ 18-22 g (start of treatment)	
Number of animals per group	10 animals/group	
Control animals	Yes	
Administration/ Exposure	Oral (diet)	
Duration of treatment	4 weeks for rat groups 1, 3, 5, 6, 7 and both mouse groups, 13 weeks for rat groups 2, 4.	
Frequency of exposure	Continuous (diet, *ad-lib*)	
Post exposure period	None	
Type	Dietary	
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
Vehicle	Acetone was used to add the test compound to the diet
Concentration in vehicle	N/A
Total volume applied	N/A
Controls	vehicle, plain Altromin 1321diet or Altromin 1321 diet + 1.25% NH_4Cl
Examinations	
Observations	
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
Mortality	Yes, observed twice daily (except weekends and holidays when animals were observed once a day)
Body weight	Yes, weekly, and prior to necropsy
Food consumption	Yes, calculated weekly
Water consumption	No
Ophthalmoscopic examination	No
Haematology	No
Clinical Chemistry	No
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. Parameters: pH and microcrystal analysis
Sacrifice and pathology	
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
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.
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. Results and Discussion
Observations	
Clinical signs	No effects
Mortality	No deaths
Body weight gain	No effects
Food consumption and compound intake	No effects
Blood analysis	
Haematology	Not assessed
Clinical chemistry	Not assessed
Urinalysis	<u>pH</u> 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). <u>Creatinine</u> 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. <u>Micro Crystals (Urinary Filters)</u> $MgNH_4PO_4$ 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. <u>Tetrafluobenzoic Acid (TFBA)</u> 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.

Sacrifice and pathology

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.

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 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). Applicant's Summary and conclusion
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).
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 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. 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.
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.
LO(A)EL	Not relevant
NO(A)EL	Not relevant
Reliability	1
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	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 Anonymous, 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)

3.9.4.4 Study 4 - Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human

Section A 6.10/04	Mechanistic Study
BPD Annex Point IIIA6.7	Comparison of the in vitro metabolism in Liverbeads™, from male rat, mouse dog and human

	Reference	Official use only
Data protection	Yes	
Data owner	Bayer CropScience AG	
Companies with letter of access	None	
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.	
	Guidelines and Quality Assurance	
Guideline study	No, no guidelines available	
GLP	Yes	

Deviations	No, there were no protocol deviations during the study MATERIALS AND MethodS
Test material	Transfluthrin [methylene-^{14}C]-Transfluthrin
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
Specification	As given in section 2
Description	Transfluthrin: white melt [methylene-^{14}C]-Transfluthrin: solid
Purity	Transfluthrin: 99.6% [methylene-^{14}C]-Transfluthrin: >99% (radio purity, HPLC), specific activity: 3.67 MBq/mg
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.
Preliminary test	**cytotoxicity evaluation**
Organism/cell type	freshly isolated rat hepatocytes
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
Controls	Positive (SDS) and negative (DMSO) controls were also assayed in this experiment.
Concentrations	In the preliminary test, 3 concentrations of Transfluthrin were tested: 25, 100 and 400 µM (two wells per concentration were used).
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
Main study	***in vitro* metabolism**
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
Source	Biopredic International, Rennes, France
Administration / Exposure; Application of test substance	Non-entry field
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
Way of application	For each species: 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).
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.
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.
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.
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) Results and Discussion
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.

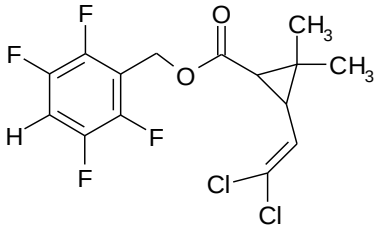
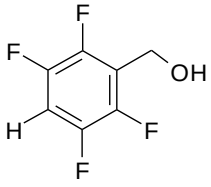
	TFBA was only a minor metabolite in the mouse (3.1%) and in the rat (0.9%), and was not detected in the two other species. After 24 hours of incubation, high amounts of glucuronide -TFB alcohol were measured in the rat (92.1%), in the mouse (93.5%) and in the dog (76.6%). By contrast, in human the level 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.
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 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%).
	Applicant's Summary and conclusion
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.
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).
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 (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%).
Reliability	1
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	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 Anonymous, 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	

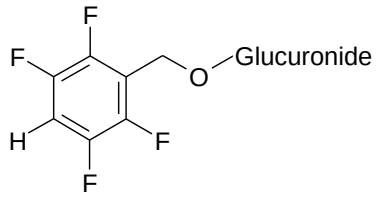
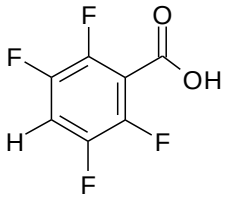
Glucoconjugated TFB alcohol, WAK5256 Original sample ID: WAK5256	Glucuronide conjugated of the 2,3,5,6-tetrafluoro-benzylalcohol	
TFBA; TFB acid, TFB-OOH, (FHW0119D) Original sample ID: 950627ELB01	2,3,5,6-tetrafluoro-benzoic acid	

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

3.9.4.5 Study 5 - The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

Section A 6.10/05	Mechanistic Study
Annex Point IIIA6.7	The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

	Reference	Official use only
Data protection	Yes	
Data owner	Bayer CropScience AG	
Companies with letter of access	None	
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.	
	Guidelines and Quality Assurance	
Guideline study	No, no guidelines available	
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.	
Deviations	No, there were no protocol deviations during the study	
	MATERIALS AND MethodS	
Test material	Transfluthrin	
	Tetrafluorobenzoic acid (TFBA)	
Lot/Batch number	Transfluthrin: Batch No. ABIDTBN019; Certificate No. AZ 15885	
	Tetrafluorobenzoic acid: Batch No. 950627ELB01; Certificate No. AZ 12737	
Specification	As given in section 2 for transfluthrin	
Description	Transfluthrin: white melt	
	Tetrafluorobenzoic acid: white crystals	
Purity	Transfluthrin: 99.6%	
	Tetrafluorobenzoic acid: 99.0%	
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.	
Preliminary test	**cytotoxicity evaluation**	
Organism/cell type	MYP3 rat urothelial cell	
	1T1 human urothelial cell line	
	Provided by Dr. Ryoichi Oyasu, Northwestern University, Chicago, U.S.A.	
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.
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.
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.
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 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. 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). Applicant's Summary and conclusion
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. 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.

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).

Conclusion

Section A 6.10/05 Annex Point IIIA6.7	Mechanistic Study The Effects of Treatment with Transfluthrin and Tetrafluorobenzoic Acid on Rat and Human Urothelial Cell Lines

Reliability	1
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	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 Anonymous, 2010. RMS supports the conclusions described in the position papers.
	COMMENTS FROM...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

3.9.4.6 Study 6 – Transfluthrin – preliminary concentration range finding study in cultured female B6C3F1 mouse hepatocytes

	Transfluthrin – preliminary concentration range finding study in cultured female B6C3F1 mouse hepatocytes

Reference	M-648001-02-1, Concept Life Sciences Study No. CLS4_0008_0002	Official use only
Data protection		
Data owner	Bayer SAS	
Companies with letter of access	none	
Criteria for data protection		
Guideline study	no specific guideline	
GLP	no	
Deviations	no	

	Transfluthrin – preliminary concentration range finding study in cultured female B6C3F1 mouse hepatocytes
Test material	Transfluthrin (NAK 4455) (AE 0035474)
Lot/Batch number	PMLO000319
Specification	
Description	Off-white solid
Purity	98.9 % w/w
Test Animals	
Species	Mouse – Female only
Strain	B6C3F1
Source	Envigo (Woolley Rd, Alconbury, Huntingdon PE28 4HS)
Sex	females
Age/weight at study initiation	8-9 weeks approximately at start of treatment
Test system	Primary monolayer cultures of hepatocytes
Study design	The aim of this study was to evaluate the potential cytotoxicity of the test compound, transfluthrin, by measuring adenosine 5´-triphosphate (ATP) levels in isolated female B6C3F1 mouse hepatocytes. The purpose of this preliminary study was to select suitable concentrations for use in future investigative studies.
Methods	A female mouse was euthanatized and hepatocytes were collected by in situ perfusion. Hepatocytes were cultured in 96-well plates and exposed to 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300 µM transfluthrin for 3 days. The experiment was repeated using higher concentrations of transfluthrin (0, 3, 10, 30, 100, 300, 500, 600, 900 and 1000 µM). Cytotoxicty was determined by measuring measuring adenosine 5'-triphosphate (ATP) concentrations by means of luminometry. Sstatistal analysis were performed using an one-way analysis of variance (ANOVA) and Dunnett's multiple comparison.
Viability	Viabilities of the hepatocyte preparation, as determined by Trypan Blue exclusion (Laboratory Method Sheet (LMS) Tic-002), were in excess of 70%.
RESULTS AND DISCUSSION	In the first test slight cytotoxicity was observed at all tested concentrations at similar levels. Therefore a second test was performed using a greater concentration range of transfluthrin. The results demonstrate no statistical significant levels of cytotoxicity. At 300 µM and above, transfluthrin was found not to completely dissolve in the culture medium which could explain the lack of cytotoxicity at the highest concentratiosn tested.
CONCLUSION	Concentrations of 30, 100, 300 and 1000 µM transfluthin were selected for the main study.

3.9.4.7 Study 7 – Transfluthrin – Enzymze, mRNA and DNA synthesis induction in cultured females B6C3F1 mouse hepatocytes

	Mechanistic Study Transfluthrin – Enzymze, mRNA and DNA synthesis induction in cultured females B6C3F1 mouse hepatocytes
Reference	M-645797-01-1, Concept Life Sciences Study No. CLS4_0008_0003
Data protection	
Data owner	Bayer SAS
Companies with letter of access	none
Criteria for data protection	
Guideline study	no specific guideline
GLP	no
Deviations	no
Test material	Transfluthrin (NAK 4455) (AE 0035474)
Lot/Batch number	PMLO000319
Specification	
Description	Off-white solid
Purity	98.9 % w/w
Test Animals	
Species	Mouse – Female only
Strain	B6C3F1
Source	Envigo (Woolley Rd, Alconbury, Huntingdon PE28 4HS)
Sex	females
Age/weight at study initiation	11-12 weeks approximately at start of treatment
Test system	Primary monolayer cultures of hepatocytes
Viability	In excess of 72% (hepatocytes from five independent perfusions were pooled) – Trypan Blue exclusion
Study design	The aim of this study was to investigate the potential for transfluthrin to activate the following nuclear hormone receptors (NHRs): aryl hydrocarbon receptor (Ahr); constitutive androstane receptor (Car); pregnane-X-receptor (Pxr) and peroxisome proliferator activated receptor alpha (Pparα) in hepatocyte cultures isolated from female B6C3F1 mice. An additional aim was to assess if transfluthrin stimulated cell proliferation (measured as a change in replicative DNA synthesis [Sphase]). NHR activation was assessed by downstream cytochrome P450 (Cyp) enzyme activity measurements and Taqman® mRNA analysis. Phenobarbital (PB) and epidermal growth factor (EGF) were included as positive control reference items. Statistical comparisons between hapatocytes treated with Test/Control item and the DMSO control group will be undertaken using a one way analysis of variance (ANOVA) followed by a Dunnett's multiple comparison. Analysis will be performed using GraphPad Prism 7 (GraphPad Software Inc., La JoMar Cat.
Methods	
Hepatocyte isolation	Five mice were terminally anaesthetised using Euthatal and hepatocytes isolated by in situ perfusion according to Mitchell A.M. et al., (1984). Viabilities of the hepatocyte preparations, as determined by Trypan Blue exclusion (Laboratory Method Sheet (LMS) Tic-002), were in excess of 72% and hepatocytes from five independent perfusions were pooled for use in the main experiment.

Official use only

Hepatocyte culture	Primary monolayer cultures of hepatocytes were established in 96-well plates (at a seeding density of 2 x 104 cells/well), 6-well plates (at a density of 0.8 x 106 cells/well) or 25 cm2 flasks (at a seeding density of 1 x 106 cells/flask). Freshly-isolated hepatocytes were initially cultured in Leibowitz CL15 medium (LMS Tic-001) for 4 hours to allow adherence to vessels. Thereafter, the medium was replaced with fresh Leibowitz CL15 medium containing PB at 2 concentrations (100 and 1000 µM), or transfluthrin at 4 concentrations, (30, 100 300 and 1000 µM) or vehicle control [0.1% v/v DMSO]. In addition, hepatocytes (5 replicates in 6-well plates) were exposed to EGF (25 ng/mL). EGF was used as a positive control for induction of replicative DNA synthesis (S-phase). The culture media, including Test/Control Items, was replenished daily. There were 6 replicates for each concentration in 96-well plates for cell toxicity (ATP) measurements, 5 replicates for each concentration in 6-well plates for replicate DNA synthesis (S-phase) analysis, 3 replicates for each concentration in 25 cm2 flasks for enzyme activity measurements and 3 replicates for each concentration in 6-well plates for mRNA analysis. The Test Item was formulated in DMSO and Test/Reference Items were administered such that the final DMSO concentration in all cultures was 0.1% (1 µL DMSO/mL medium).
Assays	After 96 hours in culture, hepatocytes were either, methanol fixed for assessment of S-phase or harvested by scraping into SET buffer (0.25 M sucrose, 5 mM EDTA, 20 mM Tris-HCl, pH 7.4) for subsequent enzyme activity analysis. The scraped mixture was sonicated and stored at approximately –70 °C until analysis. Additional cells were harvested into RLT lysis buffer, vortexed and stored at approximately -70 °C for mRNA extraction and analysis. Protein concentration was determined by the method of Lowry et al. 1951 (LMS Spec-001).
Cytotoxicity	Cell toxicity was assessed following 96 hours of culture as indicated by ATP depletion. Cellular ATP was determined by luminometry according to the SOP (LMS-Spec-009). The bioluminescent determination of ATP released from viable cells was carried out using a CellTitre-Glo luminescent cell viability assay an assay kit (Promega) according to manufacturer's instructions. Results were expressed as a percentage of the ATP released from the control cells.
Replicative DNA Synthesis (S-Phase)	The number of cells undergoing replicative DNA synthesis (S-phase) in any given cell population can be determined by measuring the incorporation of 5-bromo-2′-deoxyuridine (BrdU) in newly synthesised DNA. S-phase was determined immunocytochemically. BrdU was available to the cells for the last 3 days of culture. The number of hepatocytes undergoing DNA synthesis was recorded as the labelling index [(no. of labelled hepatocytes /total no. of hepatocytes) x 100].
Ethoxyresorufin-O-deethylation (EROD)	Ethoxyresorufin is a selective substrate for Cyp1a1. Ethoxyresorufin-O-deethylation (EROD) was determined as described by Burke et al., (1985) and according to LMS Fluor-002.
Pentoxyresorufin-O-dependentylation (PROD)	Pentoxyresorufin is a selective substrate for Cyp2b. Pentoxyresorufin-O-dependentylation (PROD) was determined as described by Burke et al., (1985) and according to LMS Fluor-002.
Benzyloxyresorufin-O-debenzylation (BROD)	Benzyloxyresorufin is a selective substrate for Cyp2b and Cyp3a. Benzyloxyresorufin-Odebenzylation (BROD) was determined as described by Burke et al., (1985) and according to LMS Fluor-002.
Benzyloxyquinoline-O-	Benzyloxyquinoline is a selective substrate for Cyp3a. Benzyloxyquinoline-

debenzylation (BQ)

O-debenzylation (BQ) was determined as described by Mayer et al., (1990) and according to LMS Fluor-005.

CN--insensitive acyl CoA oxidation

CN--insensitive acyl CoA oxidation is a marker of peroxisome proliferation and was determined using palmitoyl CoA as a substrate, as described by Bronfmann et al., (1979), and according to LMS Spec-003.

Taqman® mRNA analysis

Total RNA was extracted from cultured mouse hepatocytes according to LMS Trap-002 and cDNA was synthesised from all available RNA samples according to LMS DNAt-007. TaqMan® analysis was performed according to LMS DNAt-003 on all available samples using primers specific for Cyp1a1, Cyp1a2, Cyp2b10, Cyp3a11, Cyp4a10, Cyp4a14 and Acox1 (Assay-ondemand kits, Applied Biosystems). β-actin was used as the internal standard (Assay-ondemand kits, Applied Biosystems – see Table 1 for probe information). Data was analysed by generation of ΔCT and ΔΔCT values for all genes.

Table 1: TaqMan® Genomic Assays to be used

Probe Name	Catalogue Number
Cyp1a1	Mm00487218_m1
Cyp1a2	Mm00487224_m1
Cyp2b10	Mm00456588_mH
Cyp3a11	Mm00731567_m1
Cyp4a10	Mm01188913_g1
Cyp4a14	Mm00484132_m1
Acox1	Mm01246834_m1
B-Actin	Mm00607939_s1

RESULTS AND DISCUSSION

ATP depletion assay to assess cytotoxicity of transfluthrin or PB

Based on preliminary cytotoxicity data from a previous study (CLS4_0008_0002 – Study ReportM-648001-02-1), four concentrations of transfluthrin (30, 100, 300 and 1000 μM) were chosen to analyse potential effects of transfluthrin on mRNA expression, biochemical analyses and S-Phase. Cytotoxicity of PB was assessed in parallel. No statistical differences were observed between either transfluthrin or PB treated hepatocytes compared to DMSO control (Figure 1 and Table 2). Undissolved test item was observed in hepatocyte cultures where cells were dosed with transfluthrin at 100, 300 and 1000 μM.

Biochemical analysis

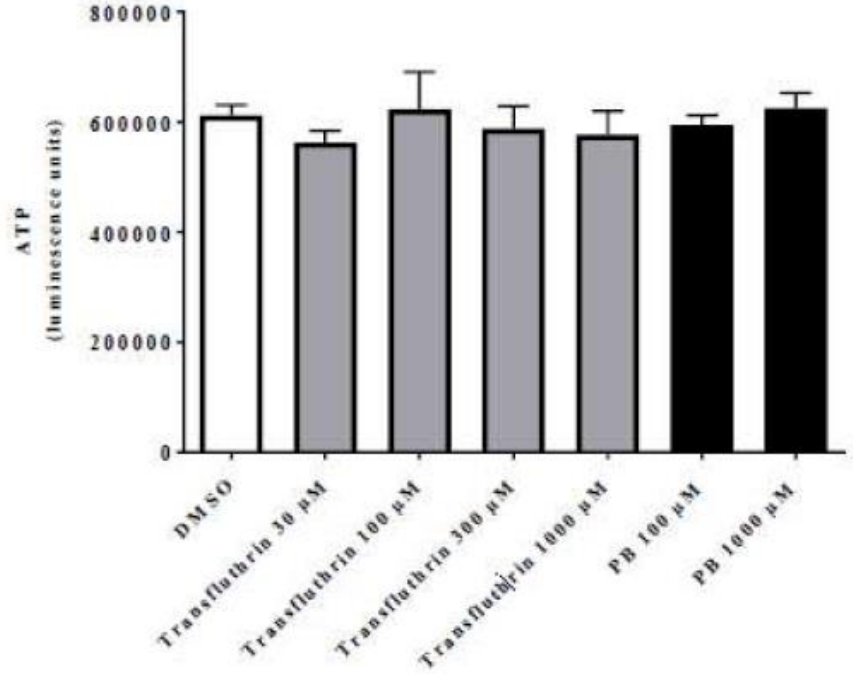
Treatment with transfluthrin resulted in statistically significant decreases in EROD, PROD, BROD and BQ activities, to 20%, or less, of control (Figure 2, Figure 3, Figure 4, Figure 5 and Table 3). Whereas, PB resulted in increases in EROD, PROD, BROD and BQ activities, to a maximum of 2.2- , 2.0-, 2.2- and 1.6-fold respectively (Figure 2, Figure 3, Figure 4, Figure 5 and Table 3). Neither transfluthrin, nor PB had an effect on CN--insensitive acyl CoA oxidation (Figure 6 and Table 3). These data suggest

	that transfluthrin may inhibit cytochrome P450 enzyme activity in cultures of primary hepatocytes isolated from female B6C3F1 mice.
Gene expression analysis	Treatment of hepatocytes with 300 µM transfluthrin resulted in statistically significant increases in expression of Cyp1a1, Cyp1a2, Cyp3a11, Cyp4a10, Cyp4a14 and Acox1 mRNA levels (Figure 7, Figure 8, Figure 10, Figure 11, Figure 12, Figure 13 and Table 4). Treatment with transfluthrin at 100, 300 and 1000 µM resulted in slight, but not statistically significant, increases in Cyp2b10 mRNA expression (Figure 9 and Table 4). Treatment with PB resulted in the expected increases in Cyp1a1, Cyp1a2, Cyp2b10, Cyp3a11 and Acox1 mRNA expression (Figure 7, Figure 8, Figure 9, Figure 10, Figure 13 and Table 4). These results suggest that transfluthrin may be weakly activating Car, Pxr and Pparα due to the slight increases observed in Cyp2b10, Cyp3a11, Cyp4a10 and Cyp4a14 mRNA expression. Although a slight induction in Cyp1a1 and Cyp1a2 mRNA levels was observed, this is not considered to be Ahr dependent. Classical activators of the Ahr, such as 2,3,7,8- tetrachlorodibenzodioxin (TCDD), induce EROD activity approximately 20-fold, (Xu et al. 2000) and this is accompanied by a marked induction of Cyp1a1 (500 fold) and, to a lesser extent, Cyp1a2 (2 fold) mRNA expression (Budinsky et al. 2010).
Replicative DNA synthesis	The positive control for replicative DNA synthesis (S-Phase), EGF, responded as expected and a statistically significant increase in replicative DNA synthesis (S-Phase) was observed, demonstrating that the hepatocytes were capable of proliferating and, were a suitable test system to investigate replicative DNA synthesis (S-Phase) in vitro. Administration of transfluthrin to these hepatocytes (at 100, 300 and 1000 µM) also resulted in increases in replicative DNA synthesis (S-Phase) of 2.2-, 2.3- and 1.8-fold increase, respectively, compared with control (Figure 14 and Table 5). However, in this instance, PB did not result in an increase in replicative DNA synthesis (S-Phase) at 100 or 1000 µM in this experiment. Previous historical data, from Concept Life Sciences, has shown that hepatocytes isolated from mice (C57 and CD-1) do respond to PB with an average increase of 1.6- fold, suggesting that mouse hepatocytes are capable of responding to PB.
CONCLUSION	This study has shown that transfluthrin does not result in cytotoxicity at any tested concentrations in hepatocytes isolated from female B6C3F1 mice. Slight precipitation of transfluthrin in hepatocyte culture medium was observed at concentrations of 300 µM and above. Although a slight induction in Cyp1a1 and Cyp1a2 mRNA levels was observed, this is not considered to be Ahr dependent. Classical activators of the Ahr, such as 2,3,7,8-tetrachlorodibenzodioxin (TCDD), induce EROD activity approximately 20-fold, (Xu et al. 2000) and this is accompanied by a marked induction of Cyp1a1 (500 fold) and, to a lesser extent, Cyp1a2 (2 fold) mRNA expression (Budinsky et al. 2010). Treatment with transfluthrin produced some increases in Cyp3a11 mRNA, but had only a small effect on Cyp2b10 mRNA expression. While this data suggests that transfluthrin may activate Pxr, in vivo studies with PB in mice lacking either Car or Pxr have demonstrated that the induction of Cyp2b10 and Cyp3a11 is predominantly due to Car, with Pxr playing only a minor role (Scheer et al. 2008). The present data thus suggests that transfluthrin may be a weak activator of both Car and Pxr. Treatment with transfluthrin also increased expression of Cyp4a10 and Cyp4a14, (Pparα target genes), but

had no effect on CN--insensitive acyl CoA oxidation. The treatment of female mouse hepatocytes with PB resulted in the appropriate responses to enzyme activation and gene expression. Transfluthrin induced replicative DNA synthesis (S-Phase) in a concentration dependant manner. EGF, the positive control for hepatocyte proliferation, resulted in a statistically significant increase in replicative DNA synthesis (S-Phase), confirming the responsiveness and suitability of the primary hepatocyte culture model for investigating replicative DNA synthesis (S-Phase). Taken together these results demonstrate that transfluthrin is a mitogenic agent in female B6C3F1 mouse hepatocytes and appears to be is a weak activator of some nuclear receptors including Car, Pxr and Pparα.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*March, 2019*
Materials and Methods	*In the study report it is indicated that Slight precipitation of transfluthrin in hepatocyte culture medium was observed at concentrations of 300 µM and above.This observation was also made in the preceeding range finding study (startinf from 300 µM and above), but information other than that precipitation was slight is not provided. The actual concentration that reaches the cells thus remains unclear. Nevertheless, considering that the trend of mRNA induction is already visible at the lower dose levels tested and that precipitation was indicated to be slight in the study report, the insolubility of transfluthrin in the culture medium is not considered to (notably) affect the study outcome.*
Results and discussion	*Treatment of the mouse hepatocytes with 30-1000 µM transfluthrin resulted in a weak induction of mRNA levels of different cytochrome P450 enzymes, at the dose of 300 µM. However, the expression of Cyp2b10, which is strongly induced by phenobarbital as well as e.g. sulfloxaflor was only mildly but not statistically significant induced by transfluthrin. And vice versa, Cyp 4a10 and Cyp 4a14 were slightly induced by 300 µM transfluthrin, but not by phenobarbital. All doses inhibited the activity of several liver enzymes (EROD, PROD, BROD, BQ) by a factor 5 to 100, in contrast to a clear induction by the positive control phenobarbital. No further analysis was conducted to explain this finding. Transfluthrin induced an increase in replicative DNA synthesis from 100 µM and above. However, the positive control phenobarbital had no effect on replicative DNA synthesis. Based on this study, no clear conclusion can be drawn on the question whether transfluthrin induces CAR activation.*
Conclusion	*The DS does not agree with the conclusion drawn by the applicant.*
Reliability	*2*
Acceptability	*acceptable*
Remarks	

Figure 1. Effect of transfluthrin and PB on ATP levels in primary mouse hepatocytes from female B6C3F1 mice



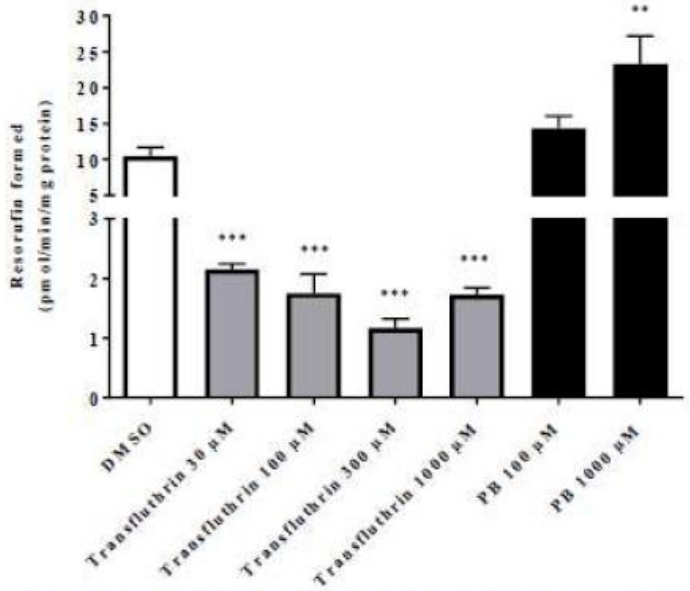
Values are Mean ± SD. n = 6 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test and no statistical differences were observed compared to DMSO control.

Table 2. Effect of transfluthrin or PB on ATP levels in primary mouse hepatocytes from female B6C3F1 mice

Test Item & Concentration	ATP content in mouse hepatocytes (luminescence units)
Vehicle control (0.1% [v/v] DMSO)	612413 ± 19098 (100.0 ± 3.1)
Transfluthrin 30 µM	562708 ± 21873 (91.9 ± 3.6)
Transfluthrin 100 µM	623115 ± 67812 (101.7 ± 11.1)
Transfluthrin 300 µM	588309 ± 41020 (96.1 ± 6.7)
Transfluthrin 1000 µM	577955 ± 42266 (94.4 ± 6.9)
PB 100 µM	594286 ± 18194 (97.0 ± 3.0)
PB 1000 µM	625695 ± 27674 (102.2 ± 4.5)

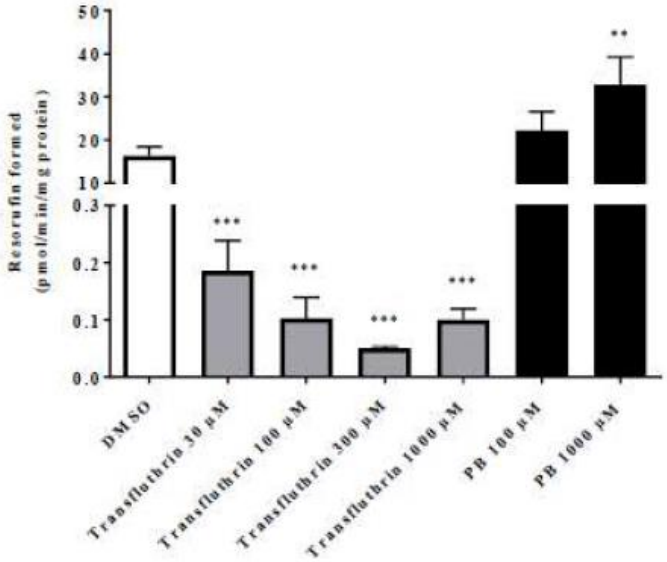
Values are Mean ± SD. n = 6 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test and no statistical differences were observed compared to DMSO control.

Figure 2. Effect of transfluthrin and PB on Ethoxyresorufin-O-deethylation (EROD) activity in primary mouse hepatocytes from female B6C3F1 mice



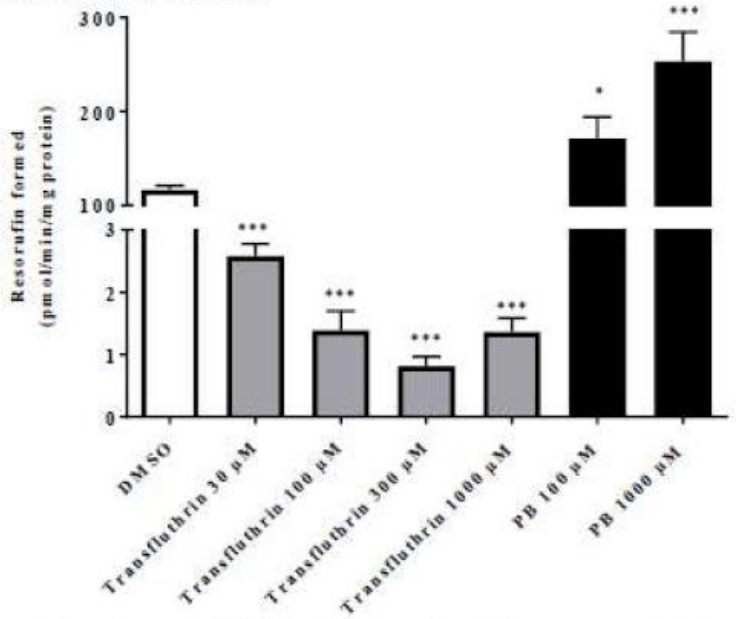
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; ** statistically different from control $p<0.01$; *** $p<0.001$.

Figure 3. Effect of transfluthrin and PB on Pentoxyresorufin-O-dependentylation (PROD) activity in primary mouse hepatocytes from female B6C3F1 mice Replicative DNA synthesis



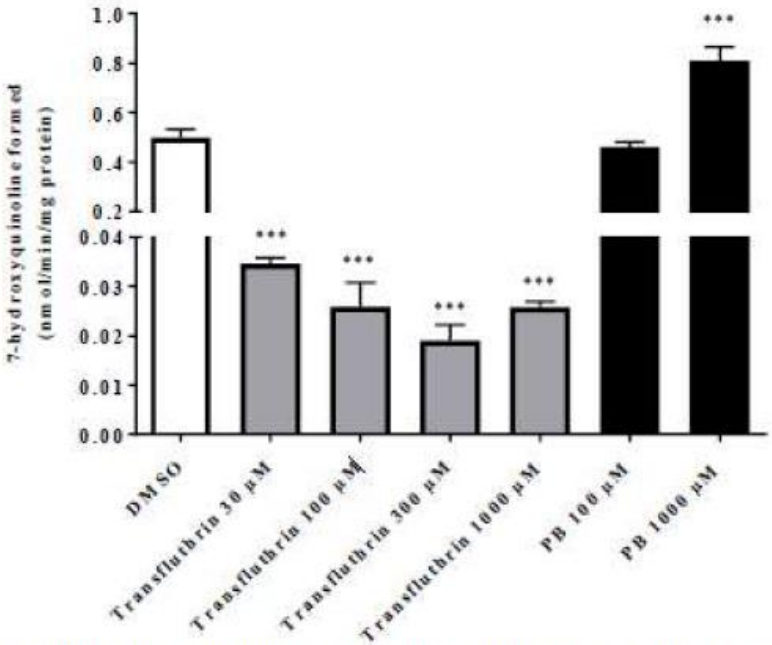
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; ** statistically different from control $p<0.01$; *** $p<0.001$.

Figure 4. Effect of transfluthrin and PB on benzyloxyresorufin-O-debenzylation (BROD) activity in primary mouse hepatocytes from female B6C3F1 mice



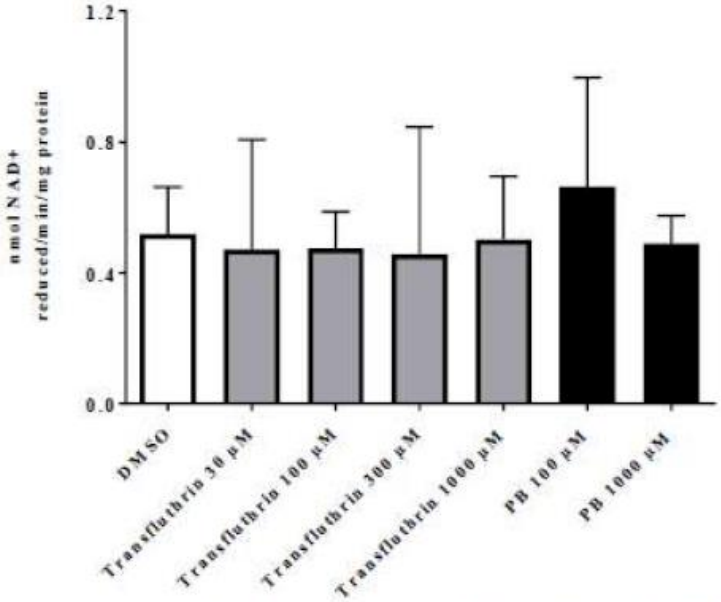
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 5. **Effect of transfluthrin and PB on benzyloxyquinoline-*O*-debenzylation (BQ) activity in primary mouse hepatocytes from female B6C3F1 mice**



Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; *** statistically different from control $p<0.001$.

Figure 6. Effect of transfluthrin and PB on CN--insensitive acyl CoA oxidation in primary mouse hepatocytes from female B6C3F1 mice



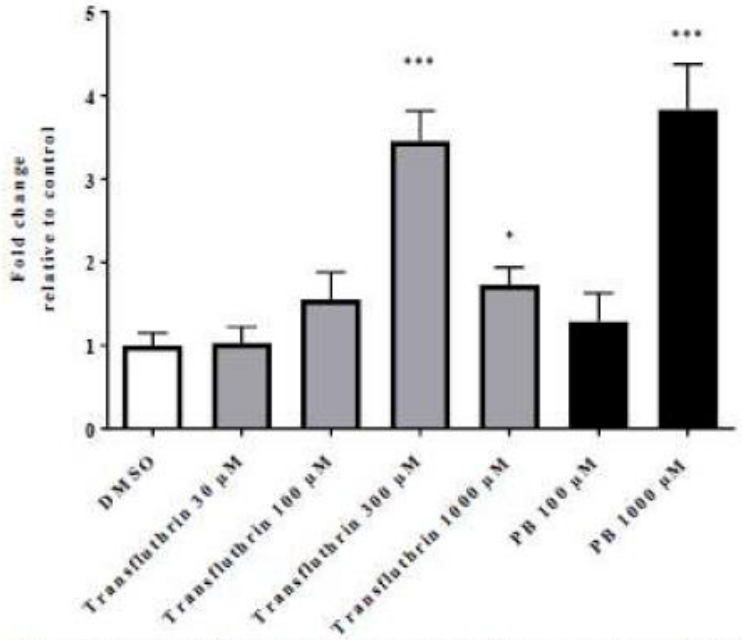
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test and no statistical differences were observed compared to DMSO control.

Table 3. **Biochemical analysis of EROD, PROD, BROD, BQ and CN^- -insensitive acyl CoA oxidation in primary mouse hepatocytes from female B6C3F1 mice**

Test Item & Concentration	EROD	PROD	BROD	BQ	CN^- - insensitive acyl CoA oxidation
	(pmol resorufin/min/mg protein)			(nmol 7-hydroxyquinoline/min/mg protein)	(nmol NAD^+ reduced/min/mg protein)
Vehicle control (0.1% [v/v] DMSO)	10.450 ± 1.272 (100.0 ± 12.2)	16.220 ± 2.094 (100.0 ± 12.9)	116.340 ± 4.752 (100.0 ± 4.1)	0.499 ± 0.034 (100.0 ± 6.8)	0.518 ± 0.143 (100.0 ± 27.7)
Transfluthrin 30 µM	2.154 ± 0.089 (20.6 ± 0.9)***	0.186 ± 0.053 (1.15 ± 0.32)***	2.575 ± 0.200 (2.21 ± 0.17)***	0.035 ± 0.001 (6.93 ± 0.24)***	0.471 ± 0.336 (90.9 ± 64.8)
Transfluthrin 100 µM	1.756 ± 0.323 (16.8 ± 3.1)***	0.102 ± 0.037 (0.63 ± 0.23)***	1.387 ± 0.310 (1.19 ± 0.27)***	0.026 ± 0.005 (5.18 ± 0.96)***	0.475 ± 0.112 (91.7 ± 21.7)
Transfluthrin 300 µM	1.172 ± 0.149 (11.2 ± 1.4)***	0.051 ± 0.003 (0.31 ± 0.02)***	0.823 ± 0.146 (0.71 ± 0.13)***	0.019 ± 0.003 (3.81 ± 0.64)***	0.457 ± 0.391 (88.2 ± 75.4)
Transfluthrin 1000 µM	1.729 ± 0.116 (16.5 ± 1.1)***	0.100 ± 0.019 (0.62 ± 0.12)***	1.363 ± 0.220 (1.17 ± 0.19)***	0.026 ± 0.001 (5.17 ± 0.23)***	0.502 ± 0.193 (96.8 ± 37.3)
PB 100 µM	14.350 ± 1.728 (137.3 ± 16.5)	22.142 ± 4.347 (136.5 ± 26.8)	170.942 ± 23.164 (146.9 ± 19.9)*	0.460 ± 0.021 (92.0 ± 4.3)	0.664 ± 0.333 (128.1 ± 64.2)
PB 1000 µM	23.323 ± 3.876 (223.2 ± 37.1)**	32.737 ± 6.541 (201.8 ± 40.3)**	253.645 ± 31.061 (218.0 ± 26.7)***	0.810 ± 0.055 (162.3 ± 11.1)***	0.490 ± 0.085 (94.6 ± 16.5)

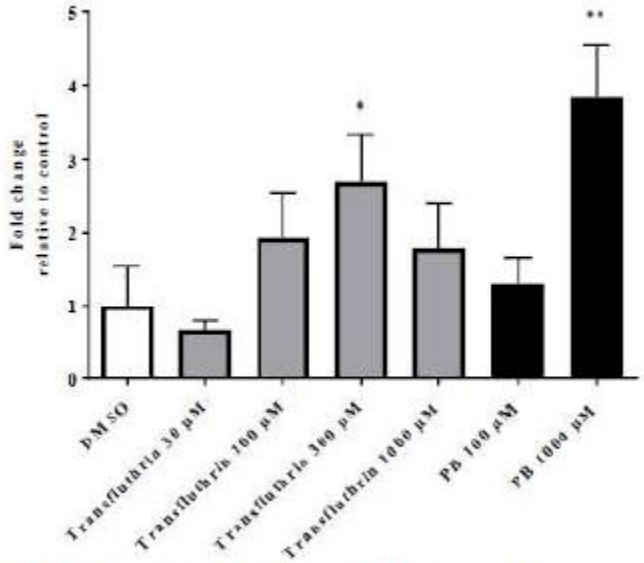
Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 3 per group. One way ANOVA was performed in the results, followed by a Dunn ett's multiple comparison test; * statistically different from control $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 7. Effect of transfluthrin and PB on *Cyp1a1* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



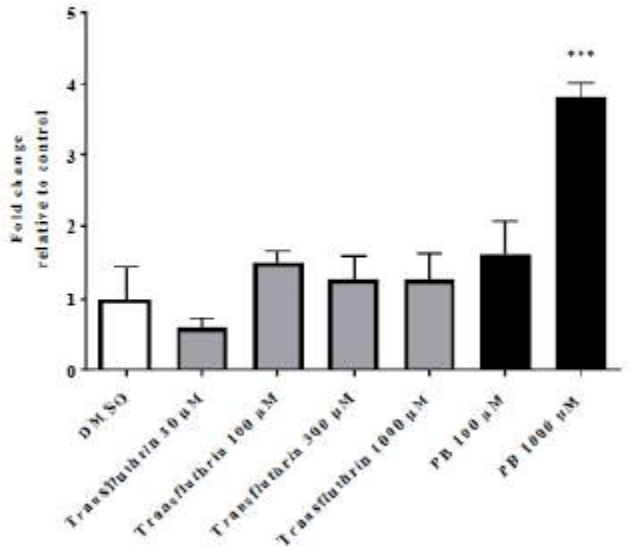
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 8. Effect of transfluthrin and PB on *Cyp1a2* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



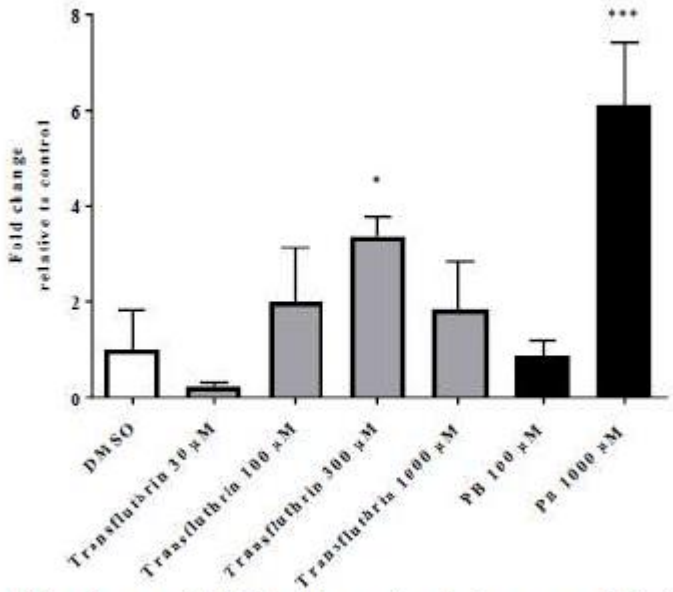
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 9. Effect of transfluthrin and PB on *Cyp2b10* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



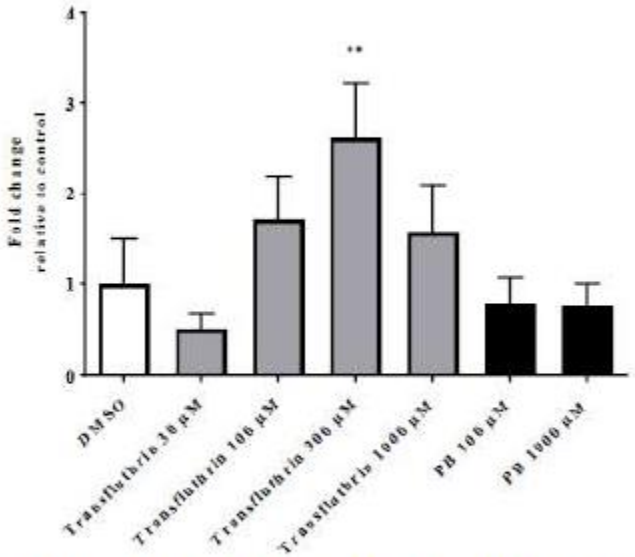
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 10. Effect of transfluthrin and PB on *Cyp3a11* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



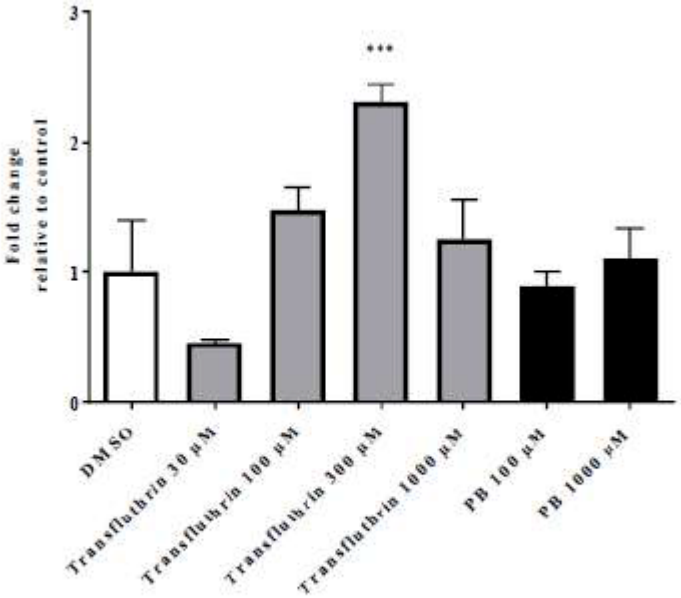
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 11. Effect of transfluthrin and PB on *Cyp4a10* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$

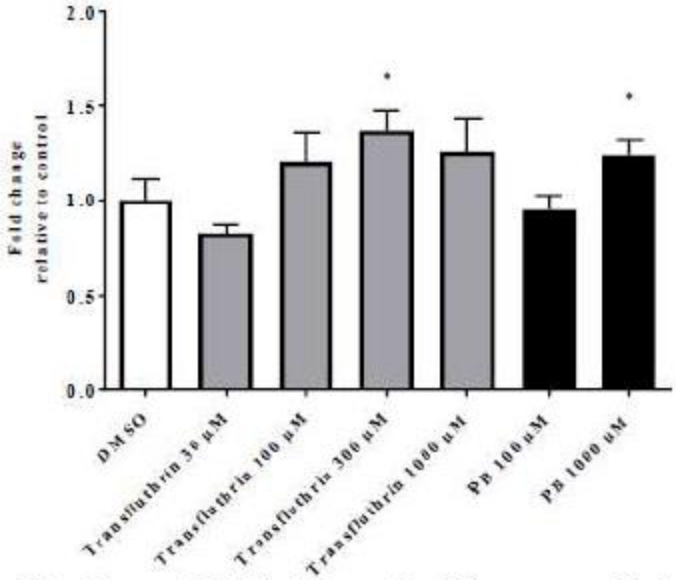
Figure 12. Effect of transfluthrin and PB on *Cyp4a14* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$.

Figure 13. Effect of transfluthrin and PB on *Acox1* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice

Figure 13. Effect of transfluthrin and PB on *Acox1* mRNA expression in primary mouse hepatocytes from female B6C3F1 mice



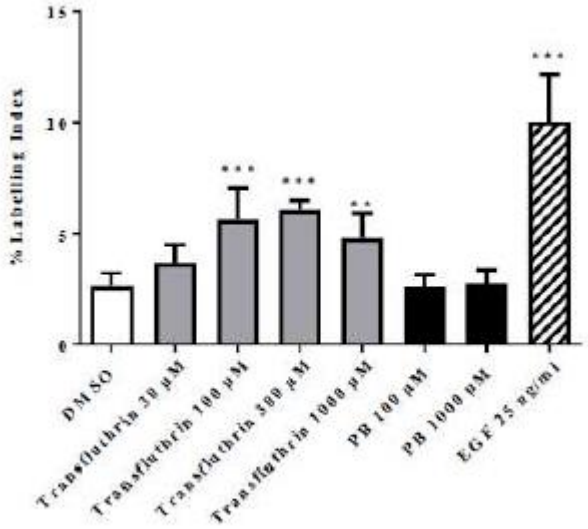
Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$

Table 4. Taqman analysis of Cyp1a1, Cyp1a2, Cyp2b10, Cyp3a11, Cyp4a10, Cyp4a14 and Acox1 mRNA in primary mouse hepatocytes from female B6C3F1 mice

Test Item & Concentration	Cyp1a1 mRNA	Cyp1a2 mRNA	Cyp2b10 mRNA	Cyp3a11 mRNA	Cyp4a10 mRNA	Cyp4a14 mRNA	Acox1 mRNA
Vehicle control (0.1% [v/v] DMSO)	1.000 ± 0.152	1.000 ± 0.543	1.000 ± 0.425	1.000 ± 0.834	1.000 ± 0.493	1.000 ± 0.398	1.000 ± 0.109
Transfluthrin 30 µM	1.031 ± 0.194	0.667 ± 0.133	0.605 ± 0.125	0.264 ± 0.070	0.522 ± 0.138	0.453 ± 0.030	0.822 ± 0.047
Transfluthrin 100 µM	1.554 ± 0.324	1.939 ± 0.616	1.490 ± 0.152	2.000 ± 1.164	1.707 ± 0.469	1.479 ± 0.171	1.207 ± 0.158
Transfluthrin 300 µM	3.456 ± 0.363***	2.697 ± 0.641*	1.285 ± 0.286	3.394 ± 0.388*	2.609 ± 0.606**	2.305 ± 0.148***	1.372 ± 0.103*
Transfluthrin 1000 µM	1.725 ± 0.210*	1.784 ± 0.609	1.280 ± 0.335	1.849 ± 1.003	1.567 ± 0.523	1.259 ± 0.296	1.258 ± 0.174
PB 100 µM	1.290 ± 0.340	1.309 ± 0.349	1.597 ± 0.464	0.870 ± 0.353	0.781 ± 0.284	0.889 ± 0.110	0.947 ± 0.075
PB 1000 µM	3.829 ± 0.543***	3.828 ± 0.712**	3.817 ± 0.202***	6.116 ± 1.322***	0.756 ± 0.246	1.095 ± 0.246	1.240 ± 0.075*

Data expressed as fold change over control. Values are Mean ± SD, n = 3 per group. One way ANOVA was performed on the results, followed by a Dunnett's multiple comparison test; * statistically different from control $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figure 14. Effect of transfluthrin, PB and EGF on replicative DNA synthesis (S-phase) in primary mouse hepatocytes from female B6C3F1 mice



Values are Mean ± SD. n = 3 per group. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; *** $p<0.001$

Table 5. Effect of transfluthrin, PB or EGF on replicative DNA synthesis (S-phase) in primary mouse hepatocytes from female B6C3F1 mice

Test Item & Concentration	S-Phase labelling index
Vehicle control (0.1% [v/v] DMSO)	2.61 ± 0.59 (100.0 ± 22.7)
Transfluthrin 30 µM	3.66 ± 0.84 (139.9 ± 32.1)
Transfluthrin 100 µM	5.63 ± 1.41 (215.6 ± 54.1)***
Transfluthrin 300 µM	6.06 ± 0.41 (231.8 ± 15.8)***
Transfluthrin 1000 µM	4.78 ± 1.13 (182.9 ± 43.4)**
PB 100 µM	2.58 ± 0.56 (98.6 ± 21.3)
PB 1000 µM	2.75 ± 0.59 (105.3 ± 22.5)
EGF [Δ] 25ng/ml	10.00 ± 2.16 (382.6 ± 82.8)***

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 5 per group except for EGF treated group (n = 4). One way ANOVA was performed on the results, followed by a Dunnett's multiple comparison test; ** statistically different from control $p < 0.01$; *** $p < 0.001$. ΔAn unpaired t test (two-tailed) was performed on the results for EGF treated hepatocytes;*** statistically different from control $p < 0.001$.

3.9.4.8 Study 8 – Transfluthrin – preliminary concentration range finding study in cultured human hepatocytes from three different cultures

	Transfluthrin – preliminary concentration range finding study in cultured human hepatocytes from three different cultures	Official use only
Reference	M-648001-02-1, Concept Life Sciences Study CLS4_0008_0004	
Data protection		
Data owner	Bayer SAS	
Companies with letter of access	none	
Criteria for data protection		
Guideline study	no specific guideline	
GLP	no	
Deviations	no	
Test material	Transfluthrin (NAK 4455) (AE 0035474)	
Lot/Batch number	PMLO000319	
Specification		
Description	Off-white solid	
Purity	98.9 % w/w	
Test Animals		
Species	Human	
Source	Corning Life Sciences, Fogostraat 12, 1060 LJ Amsterdam, Netherlands (donor number 314A) or Life Technologies, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR (donors 8210, 8239, 1765 and 1951).	
Sex	Females	
Test system	Primary monolayer cultures of hepatocytes	
Study design	The aim of this study was to evaluate the potential cytotoxicity of the test compound, transfluthrin, by measuring adenosine 5´-triphosphate (ATP) levels in isolated cultured human hepatocyte cultures from three different individuals. The purpose of this preliminary study was to select suitable concentrations for use in future investigative studies.	
Methods	Hepatocytes were cultured in collagen coated 96-well plates and exposed to 0.03, 0.1, 0.3, 1, 3, 10, 30, 100 and 300 µM transfluthrin for 3 days. Cytotoxicty was determined by measuring adenosine 5'-triphosphate (ATP) concentrations by means of luminometry. Sstatistal analysis were performed using an one-way analysis of variance (ANOVA) and Dunnett's multiple comparison. Initially, data was generated using hepatocytes from a male donor. However, it was decided to include only include hepatocytes from female donors and therefore hepatocytes from donor 314A were included instead.	
RESULTS AND DISCUSSION	The results demonstrate a statistical significant levels of cytotoxicity in two donors at transfluthrin levels of 300 µM. ATP levels were found to be 62.7% and 75.6% of the control for these two donors. In the third donor a decrease in ATP was observed but this was not statistically different from the control.	
CONCLUSION	Concentrations of 3, 10, 30 and 100 µM transfluthin were selected for the main study.	

3.9.4.9 Study 9 – Transfluthrin – Enzymze, mRNA and DNA synthesis induction in cultured females human hepatocytes

	Mechanistic Study Transfluthrin – Enzymze, mRNA and DNA synthesis induction in cultured females human hepatocytes	
Reference	M-645797-01-1, Concept Life Sciences Study No. CLS4_0008_0007	Official use only
Data protection		
Data owner	Bayer SAS	
Companies with letter of access	none	
Criteria for data protection		
Guideline study	no specific guideline	
GLP	no	
Deviations	no	
Test material	Transfluthrin (NAK 4455) (AE 0035474)	
Lot/Batch number	PMLO000319	
Specification		
Description	Off-white solid	
Purity	98.9 % w/w	
Test system		
Species	Primary female human hepatocytes from three independent donors, plateable cryopreserved	
Source	Corning Life Sciences, Fogostraat 12, 1060 LJ Amsterdam, Netherlands (donor number 314A) or Life Technologies, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR, UK (donors 1765 and 1951).	
Sex	females	
Study design	The aim of this study was to investigate the potential for transfluthrin to activate the following nuclear hormone receptors (NHRs): aryl hydrocarbon receptor (Ahr); constitutive androstane receptor (Car); pregnane-X-receptor (Pxr) and peroxisome proliferator activated receptor alpha (Pparα) in hepatocyte cultures isolated from three independent human female donors. An additional aim was to assess if transfluthrin stimulated cell proliferation (measured as a change in replicative DNA synthesis [S-phase]) in human hepatocytes. NHR activation was assessed by downstream cytochrome P450 (Cyp) enzyme activity measurements and Taqman® mRNA analysis. Statistical comparisons between hapatocytes treated with Test/Control item and the DMSO control group was undertaken using a one way analysis of variance (ANOVA) followed by a Dunnett's multiple comparison. Analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., La Jolla, Mar CA, USA).	
Methods		
Primary hepatocytes	Primary hepatocytes from three independent female human donors were assessed during the course of this study. Hepatocytes were sourced from either Corning Life Sciences, Fogostraat 12, 1060 LJ Amsterdam, Netherlands (donor number 314A) or Life Technologies, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR, UK (donors 1765 and 1951).	
Hepatocyte culture	Primary monolayer cultures of hepatocytes were established in collagen	

	coated 96-well plates (at a seeding density of 4 x 104 cells/well), collagen coated 6-well plates (at a density of 1.6 x 106 cells/well) or collagen coated 25 cm2 flasks (at a seeding density of 3.2 x 106 cells/flask). Human hepatocytes were resuscitated in Cryopreserved Hepatocytes Recovery Medium (Life Technologies, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR), then cultured in Cryopreserved Hepatocytes Plating Medium (Life Technologies, 7 Kingsland Grange, Warrington, Cheshire, WA1 4SR) in a humidified incubator at 37 °C under an atmosphere of 95 % air/5 % CO2, for approximately 6 hours to allow adherence. Thereafter, the medium was replaced with fresh Leibowitz HCL15 medium (SOP: CLS4_HEP_002_00) containing Test Item at 4 concentrations, (3, 10, 30 and 100 µM), to WY 14643 at 2 concentrations (50 and 100 µM) and a vehicle control [0.1% v/v DMSO]. These were then cultured in a humidified incubator at 37 °C under air. In addition, hepatocytes (5 replicates in collagen coated 6-well plates) were exposed to EGF (25 ng/mL). EGF was used as a positive control for induction of replicative DNA synthesis (S-phase). The culture media, including Test/Control Items, was replenished daily. There were 6 replicates for each concentration in 96-well plates for cell toxicity (ATP) measurements, 5 replicates for each concentration in 6-well plates for replicate DNA synthesis (S-phase) analysis and 3 replicates for each concentration in 25 cm2 flasks for enzyme activity measurements and 3 replicates for each concentration in 6-well plates for mRNA analysis. The Test Item was formulated in DMSO and administered such that the final DMSO concentration in all cultures was 0.1% (1 µL DMSO/mL medium). The dosing solutions were prepared at the start of the study and used for the duration of the study.
Assays	After 96 hours in culture, hepatocytes were either, methanol fixed for assessment of S-phase or harvested by scraping into SET buffer (0.25 M sucrose, 5 mM EDTA, 20 mM Tris-HCl, pH 7.4) for subsequent enzyme activity analysis. The scraped mixture was sonicated and stored at approximately –70 °C until analysis. Additional cells were harvested into RLT lysis buffer, vortexed and stored at approximately -70 °C for mRNA extraction and analysis. Protein concentration was determined by the method of Lowry et al. 1951 (LMS Spec-001).
Cytotoxicity	Cell toxicity was assessed following 96 hours of culture as indicated by ATP depletion. Cellular ATP was determined by luminometry according to the SOP (LMS-Spec-009). The bioluminescent determination of ATP released from viable cells was carried out using a CellTitre-Glo luminescent cell viability assay an assay kit (Promega) according to manufacturer's instructions. Results were expressed as a percentage of the ATP released from the control cells.
Replicative DNA Synthesis (S-Phase)	The number of cells undergoing replicative DNA synthesis (S-phase) in any given cell population can be determined by measuring the incorporation of 5-bromo-2′-deoxyuridine (BrdU) in newly synthesised DNA. S-phase was determined immunocytochemically. BrdU was available to the cells for the last 3 days of culture. The number of hepatocytes undergoing DNA synthesis was recorded as the labelling index [(no. of labelled hepatocytes /total no. of hepatocytes) x 100].
Benzyloxyquinoline-O-debenzylation (BQ)	Benzyloxyquinoline is a selective substrate for Cyp3a. Benzyloxyquinoline-O-debenzylation (BQ) was determined as described by Mayer et al., (1990) and according to LMS Fluor-005.
CN--insensitive acyl CoA	CN--insensitive acyl CoA oxidation is a marker of peroxisome proliferation

oxidation	and was determined using palmitoyl CoA as a substrate, as described by Bronfmann et al., (1979), and according to LMS Spec-003.
12-hydroxylauric acid formation (LAH)	The activity of CYP4A in cultured hepatocytes was measured as the formation of 12-hydroxylauric acid from lauric acid using LC-MS/MS detection, according to LMS MS-007.
Taqman® mRNA analysis	Total RNA was extracted from cultured mouse hepatocytes according to LMS Trap-002 and cDNA was synthesised from all available RNA samples according to LMS DNAt-007. TaqMan® analysis was performed according to LMS DNAt-003 on all available samples using primers specific for Cyp1a1, Cyp1a2, Cyp2b10, Cyp3a11, Cyp4a10, Cyp4a14 and Acox1 (Assay-ondemand kits, Applied Biosystems). β-actin was used as the internal standard (Assay-ondemand kits, Applied Biosystems – see Table 1 for probe information). Data was analysed by generation of ΔCT and ΔΔCT values for all genes.

Table 1: TaqMan® Genomic Assays to be used

Probe Name	Catalogue Number
CYP1A1	Hs01054796_g1
CYP1A2	Hs00167927_m1
CYP2B6	Hs04183483_m1
CYP3A4	Hs00604506_m1
CYP4A11	Hs00167961_m1
ACOX1	Hs01074241_m1
B-Actin	Hs99999903_m1

RESULTS AND DISCUSSION

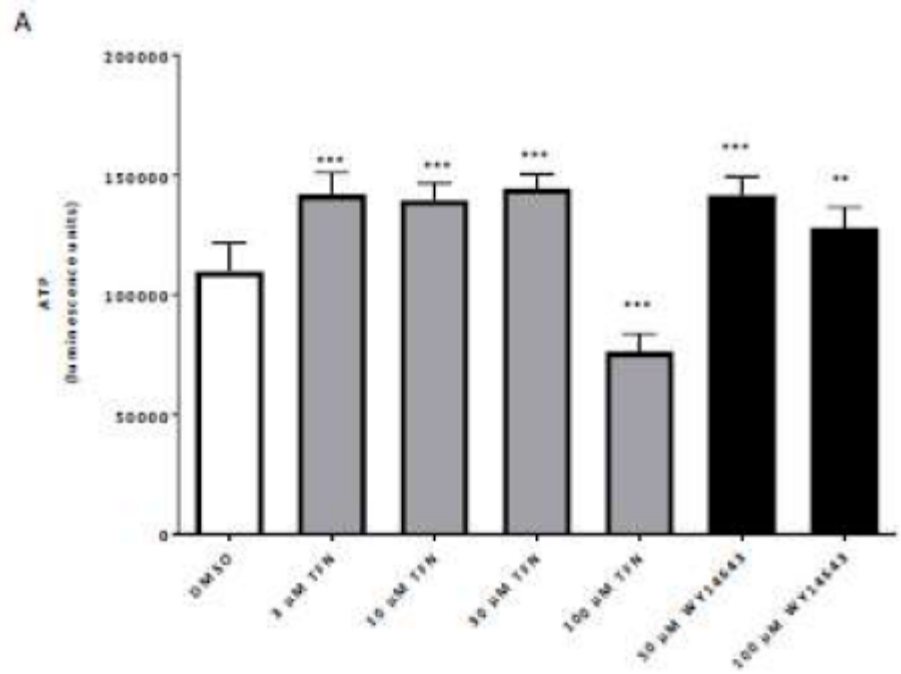
ATP depletion assay to assess cytotoxicity of transfluthrin or PB	Based on preliminary cytotoxicity data from a previous study (CLS4_0008_0004 –Study Report M-645754-01-1), four concentrations of transfluthrin (3, 10, 30 and 100 µM) were chosen to analyse potential effects of transfluthrin on mRNA expression, biochemical analyses and S-Phase. Cytotoxicity of WY 14643 was assessed in parallel. Cytotoxicity was observed at 100 µM transfluthrin in all three female human donors analysed (Figure 1 and Table 2). Cytotoxicity was not observed at any other transfluthrin or WY 14643 concentration used.
Biochemical analysis	Transfluthrin administration resulted in statistically significant, dose dependent, decreases in BQ activities reaching 27.7%, 32.3% and 46.2% of control at 100 µM transfluthrin for donors 314A, 1765 and 1951 respectively (Figure 2, Table 3, Table 4 and Table 5). While variable, no statistically significant changes in Palmitoyl CoA oxidation in donors 314A or 1951 was observed, but a decrease was observed at the highest concentration of transfluthrin assessed with a reduction in Palmitoyl CoA oxidation to 21% of control (Figure 3, Table 3, Table 4 and Table 5). Small but statistically significant increases in 12-hydroxy lauric acid formation were observed after administration with 30 and 100 µM transfluthrin (donor

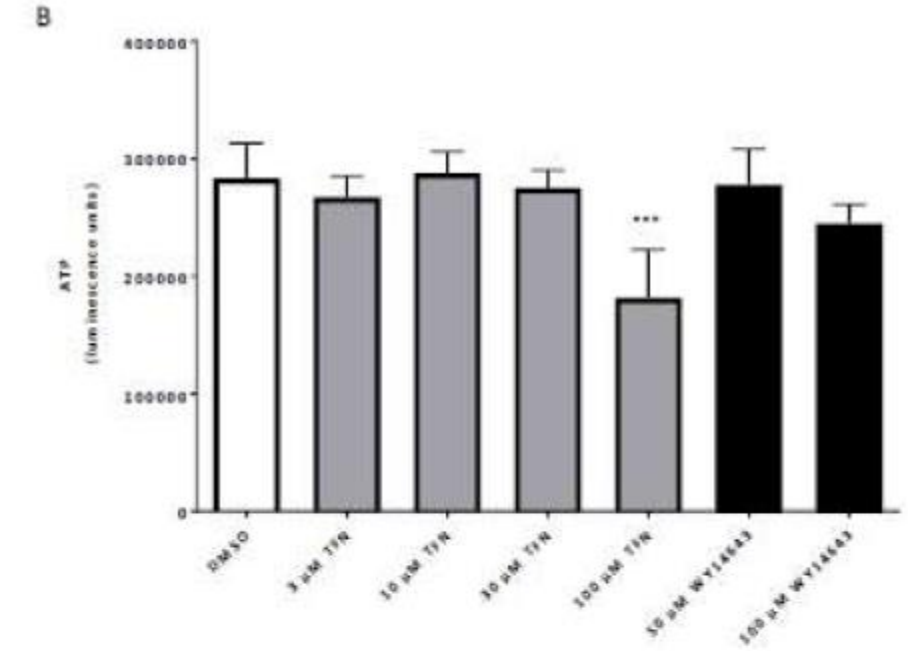
	1765, 1.2-fold) and 10 µM transfluthrin (donor 1951, 1.3-fold), no statistically significant changes were observed with donor 314A (Figure 4, Table 3, Table 4 and Table 5). Treatment with WY 14643 increased BQ activities to a maximum of 1.5-, 3.0- and 2.9-fold compared to control for donors 314A, 1765 and 1951 respectively (Figure 2). A statistically significant decrease in oxidation of Palmitoyl CoA was observed after treatment with 50 µM WY 14643 in donor 1765 (Figure 3) and a statistically significant increase in 12-hydroxy lauric acid formation was observed after treatment with 100 µM WY 14643 but only in donor 1951 (Figure 4).
Gene expression analysis	Treatment of hepatocytes with 100 µM transfluthrin resulted in a statistically significant (4-fold) induction of CYP3A4 mRNA levels for donor 314A (Figure 8 and Table 6), however, there was no increase in the other donors assessed. Treatment with transfluthrin did not result in any changes in CYP1A2, CYP2B6, CYP4A11 or ACOX1 mRNA expression (Figure 5, Figure 7, Figure 9 and Figure 10). A statistically significant decrease in CYP1A2 mRNA expression was observed after treatment with 100 µM transfluthrin in donors 314A and 1951 (Figure 6). WY 14643 administration caused variable induction in CYP mRNA levels. All six markers investigated were induced, but not in all donors, see Figure 5, Figure 6, Figure 7, Figure 8, Figure 9 and Figure 10 for details.
Replicative DNA synthesis	The positive control for replicative DNA synthesis (S-Phase), EGF, responded as expected for donors 314A, 1765 and 1951 and a statistically significant increase in replicative DNA synthesis (S-Phase) was observed (11.4-, 11.9- and 7.0-fold, respectively), demonstrating that the hepatocytes were capable of proliferating and, were a suitable test system to investigate replicative DNA synthesis (SPhase) in vitro. Administration of transfluthrin to female human hepatocytes did not result in any increases in replicative DNA synthesis (S-Phase) compared with control in donors 314A, 1765 or 1951 investigated (Figure 11 and Table 9). As expected, WY 14643 did not result in an increase in replicative DNA synthesis (S-Phase) at either 50 or 100 µM.
CONCLUSION	Transfluthrin was cytotoxic to human hepatocytes at 100 µM in all three female human donors that were investigated. Dose dependent decreases in BQ activities were observed in all three female human donors that were investigated suggesting that transfluthrin inhibited CYP3A activity. Variable effects between donors were observed with gene expression and enzyme activity across the three donors investigated. Induction of CYP3A mRNA was increased compared to control for donor 314A suggesting that transfluthrin may be a weak PXR activator in human hepatocytes but this was not consistent across all 3 donors. Transfluthrin was shown not to induce replicative DNA synthesis in female human hepatocytes, whilst the positive control, EGF, did induce replicative DNA synthesis. In summary, the data obtained does not provide any conclusive evidence that transfluthrin is a nuclear hormone receptor activator in female human hepatocytes. However, it has been shown that transfluthrin is not a mitogen in female human hepatocytes.

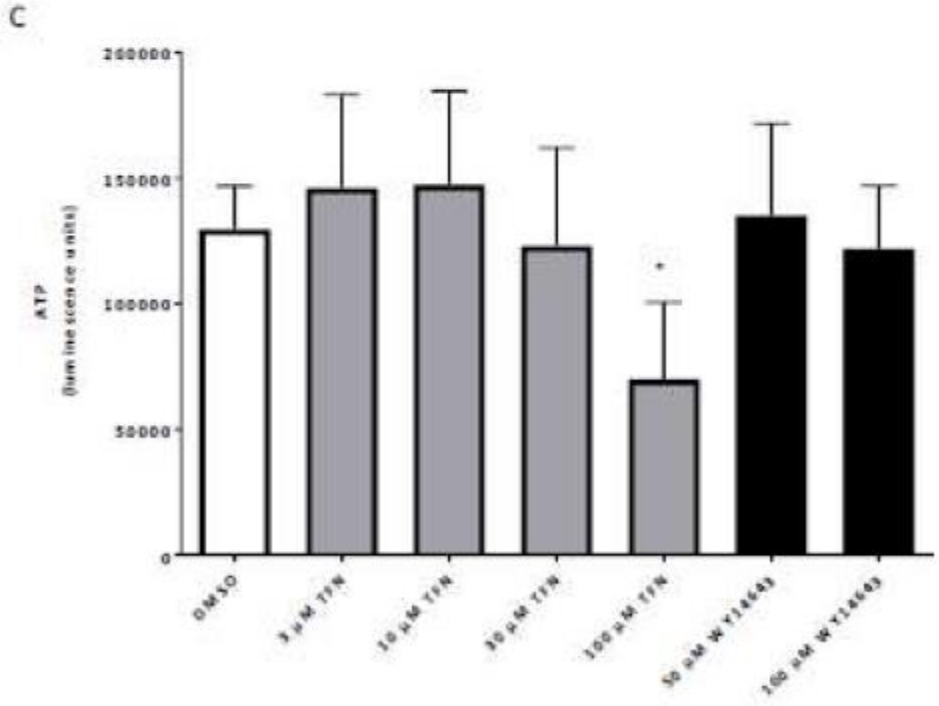
Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the

	comments and views submitted
	Evaluation by Rapporteur Member State
Date	*March, 2019*
Materials and Methods	
Results and discussion	
Conclusion	*The DS agrees with the conclusion that the results on cytochrome P450 induction are inconclusive Treatment of the human hepatocytes resulted in a dose-dependent inhibition of the liver enzyme BG (others not tested), which was induced by the positive control WY14643 (PPARα activator). The effects on the expression of mRNA of human CYP enzymes was generally small and showed high variation both between donors and samples from the same donor. Most notable was CYP3A4, which was induced in one donor but not in the others. There was no effect on replicative DNA synthesis.*
Reliability	*2*
Acceptability	*acceptable*
Remarks	

Figure 1. Effect of transfluthrin or WY 14643 on ATP levels in primary female human hepatocytes







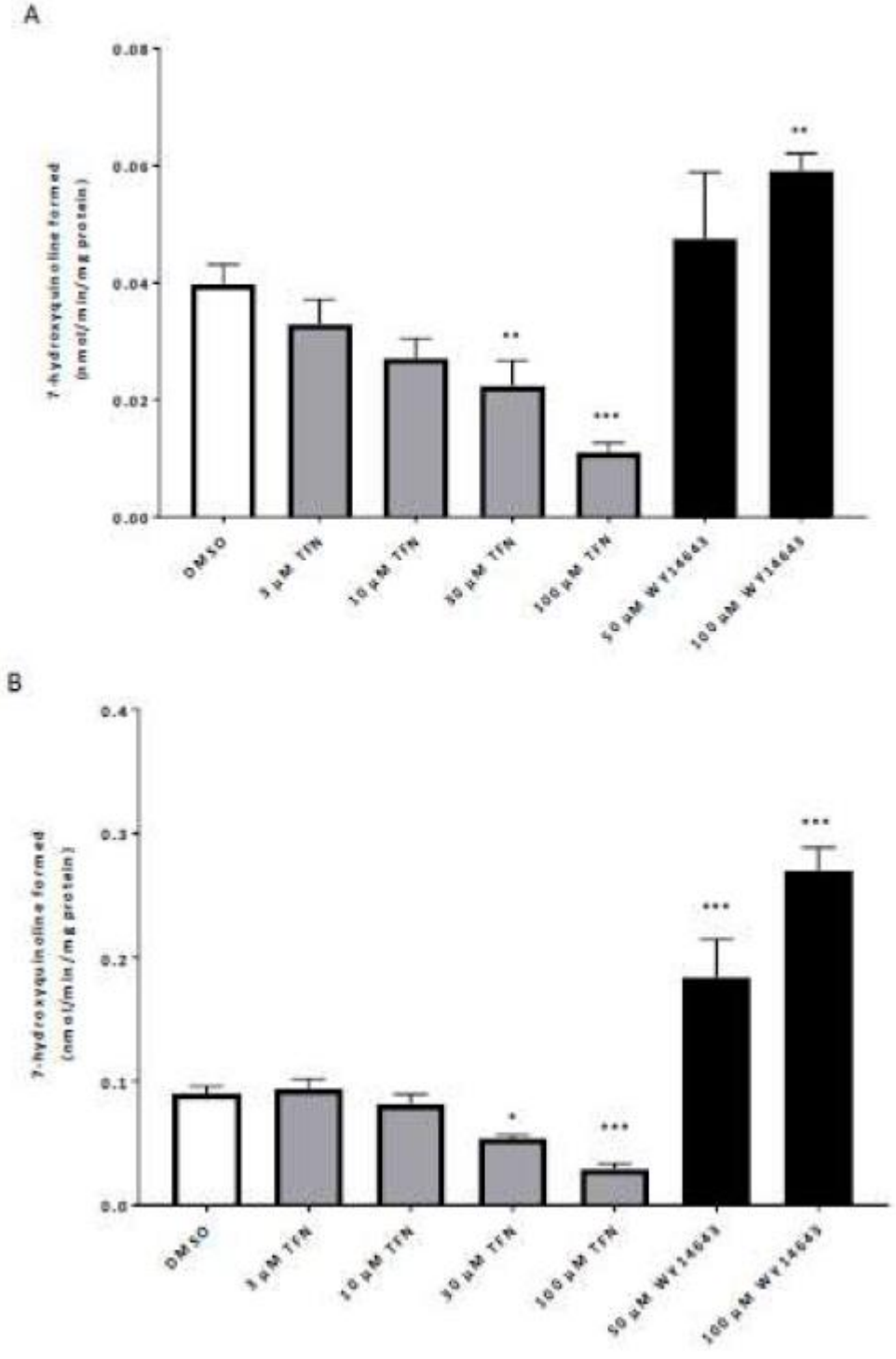
Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Table 2. Effect of transfluthrin or WY 14643 on ATP levels in primary female human hepatocytes

Test Item & Concentration	ATP content (luminescence units)		
	Donor 314A	Donor 1765 Δ	Donor 1951
Vehicle control (0.1% [v/v] DMSO)	109790 ± 11997 (100.0 ± 10.9)	283136 ± 29854 (100.0 ± 10.5)	129695 ± 17099 (100.0 ± 13.2)
WY 14643 50 µM	141683 ± 7609 (129.0 ± 6.9)***	277859 ± 30365 (98.1 ± 10.7)	135028 ± 36709 (104.1 ± 28.3)
WY 14643 100 µM	127862 ± 8710 (116.5 ± 7.9)**	245061 ± 16069 (86.6 ± 5.7)	121926 ± 25130 (94.0 ± 19.4)
Transfluthrin 3 µM	141918 ± 9190 (129.3 ± 8.4)***	267098 ± 18227 (94.3 ± 6.4)	146057 ± 37191 (112.6 ± 28.7)
Transfluthrin 10 µM	139485 ± 7070 (127.0 ± 6.4)***	287615 ± 18426 (101.6 ± 6.5)	147351 ± 37279 (113.6 ± 28.7)
Transfluthrin 30 µM	144403 ± 5991 (131.5 ± 5.5)***	275155 ± 14861 (97.2 ± 5.2)	123074 ± 39032 (94.9 ± 30.1)
Transfluthrin 100 µM	76220 ± 7349 (69.4 ± 6.7)***	181648 ± 41064 (64.2 ± 14.5)***	69990 ± 30716 (54.0 ± 23.7)*

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 6 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test; * statistically different from control $p<0.05$; **, $p<0.01$; ***, $p<0.001$ Δ For donor 1765, n = 4 for vehicle control and n = 5 for all other treatment groups.

Figure 2. Effect of transfluthrin or WY 14643 on benzyloxyquinoline-*O*-debenzylation (BQ) activity in primary female human hepatocytes



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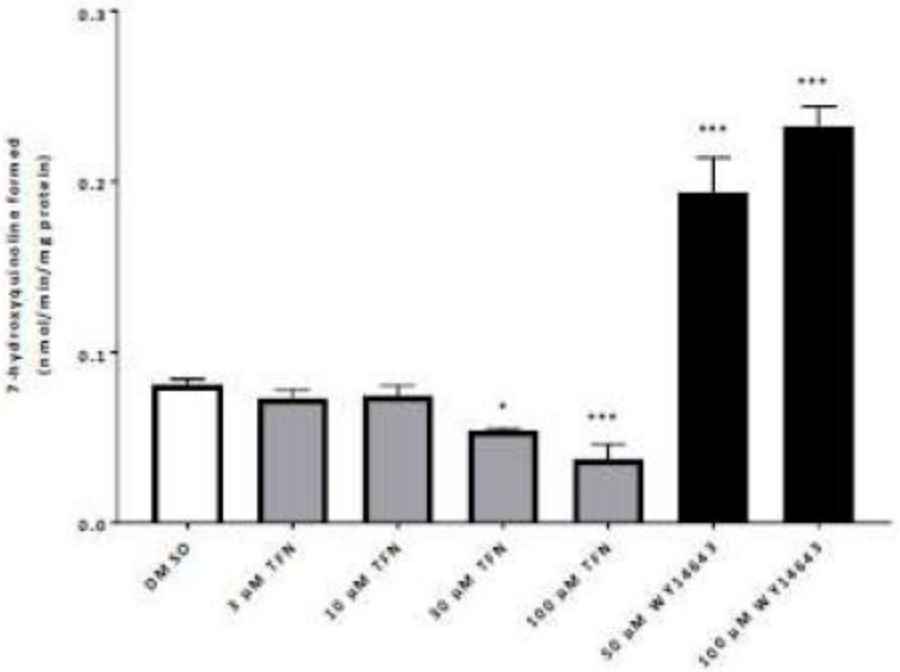
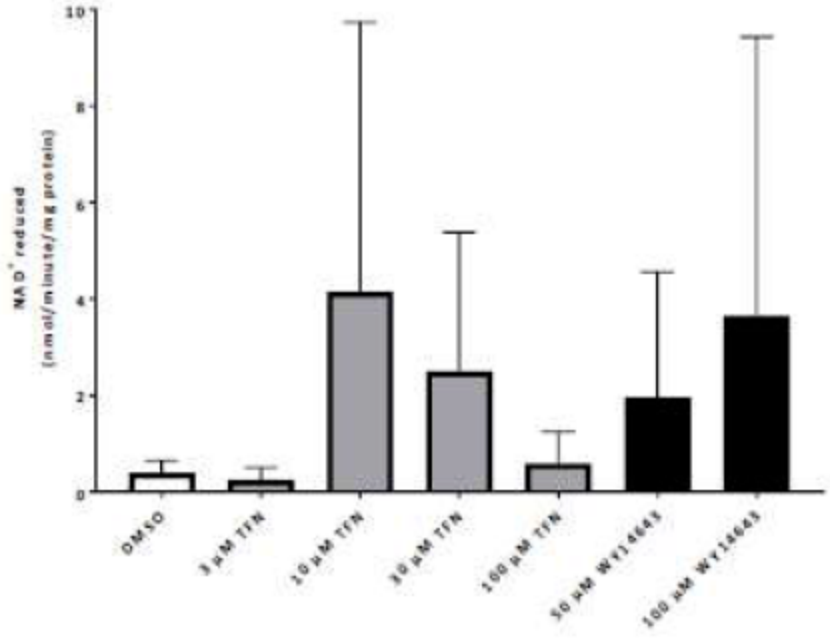


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was erformed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Figure 3. Effect of transfluthrin or WY 14643 on CN--insensitive acyl CoA oxidation in primary female human hepatocytes

A



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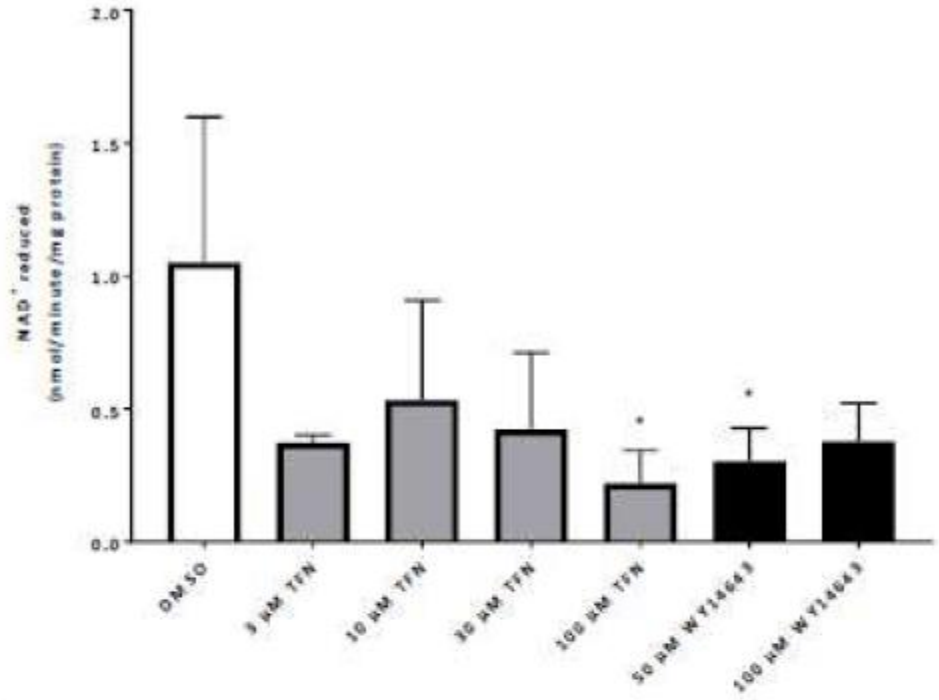

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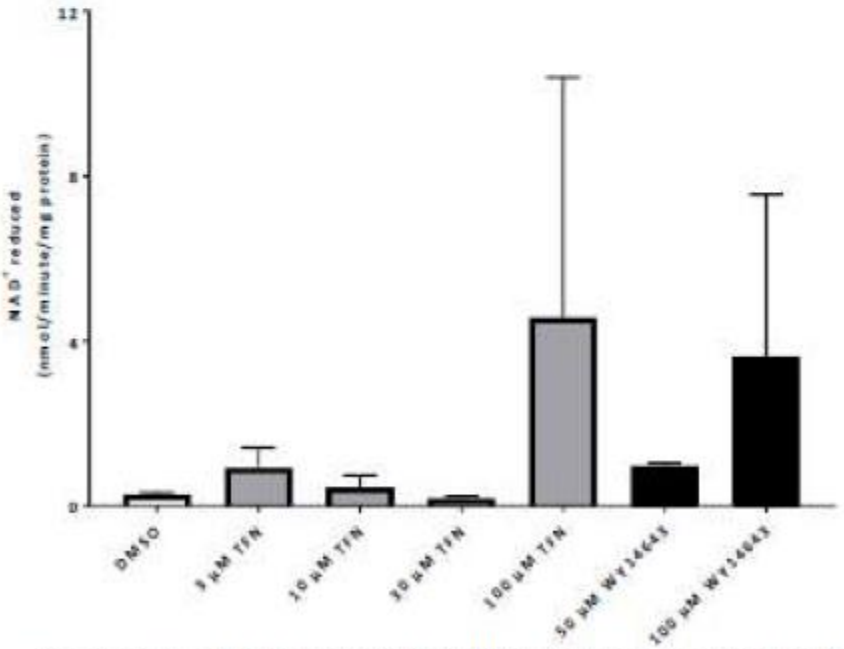
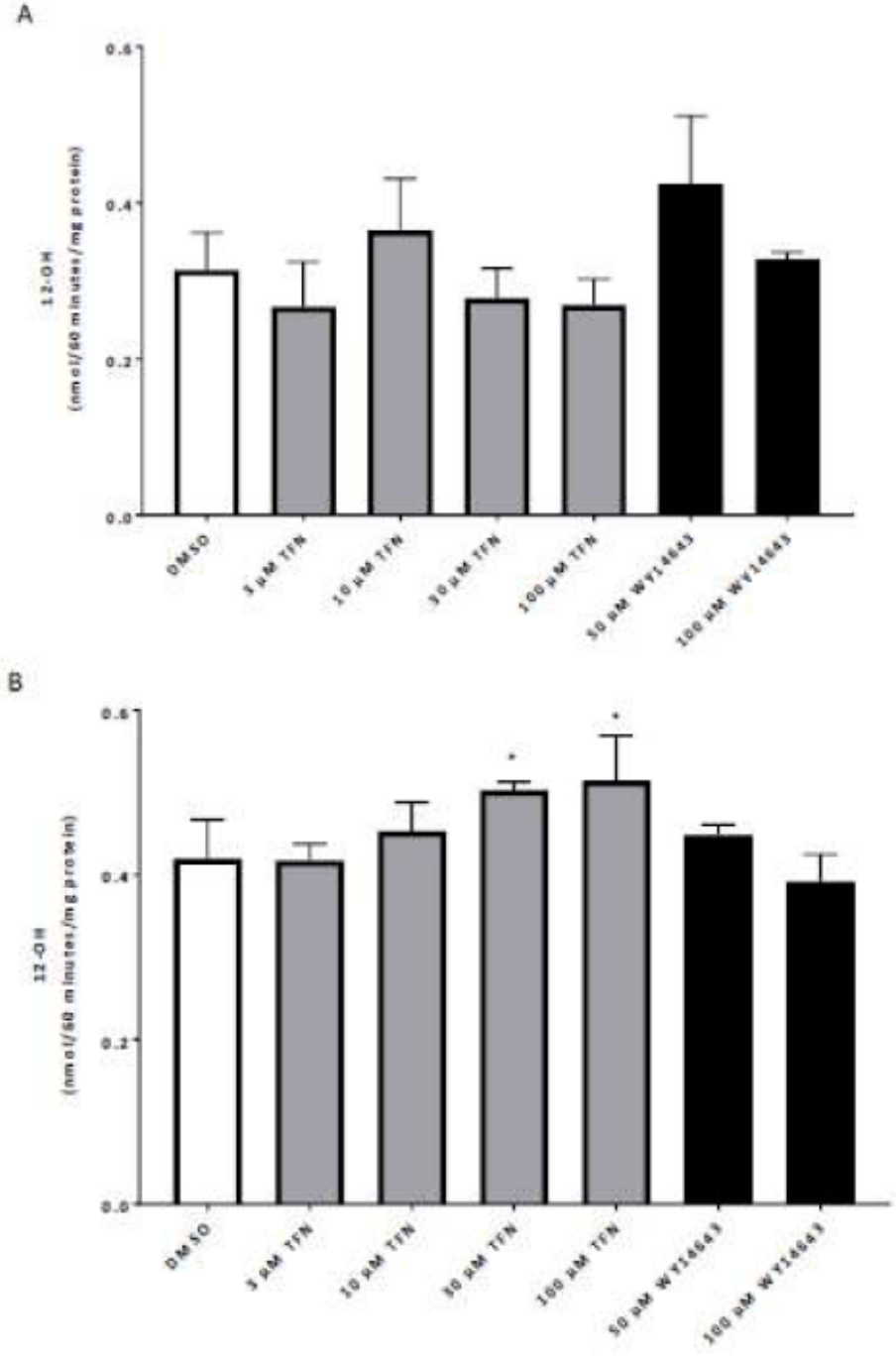
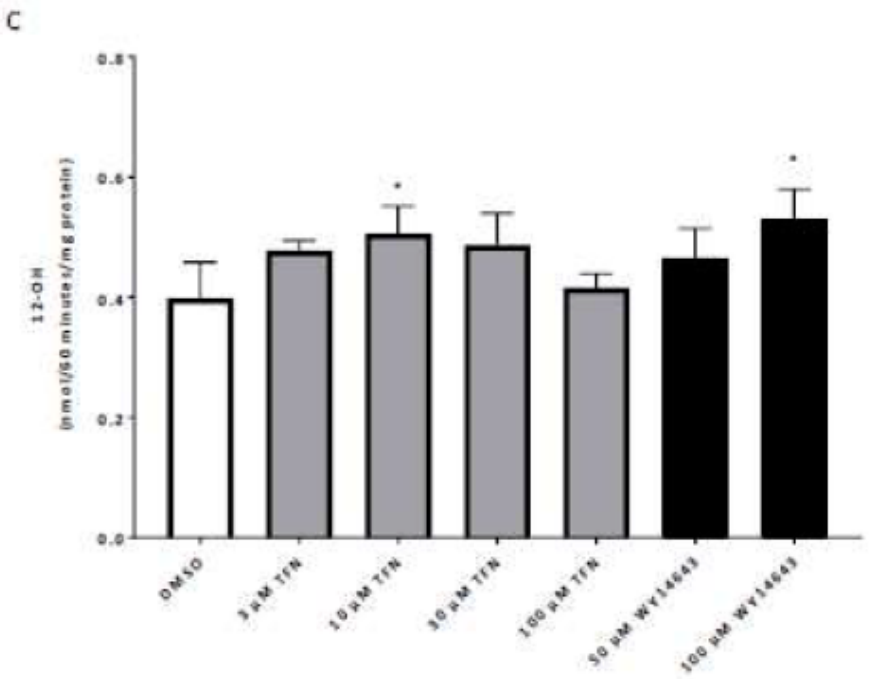


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, n = 2 for treatment groups with transfluthrin at 10µM, 30µM and 100µM, (B) Donor 1765, (C) Donor 1951 n = 2 for treatment groups with transfluthrin at 3µM, 30µM, 100µM and with WY 14643 at 50µM and 100µM. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically differen from control $p<0.05$.

Figure 4. Effect of transfluthrin or WY 14643 on 12-OH Lauric acid formation in primary female human hepatocytes





Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$.

Table 3. Biochemical analysis of BQ, CN^--insensitive acyl CoA oxidation and 12-OH Lauric acid formation in primary female human hepatocytes (donor 314A)

Test Item & Concentration	Benzyloxyquinoline-*O*-debenzylation (nmol 7-hydroxyquinoline formed /minute/mg protein)	CN^--insensitive acyl CoA oxidation$^{\Delta}$ (nmol NAD^+ reduced /minute/mg protein)	12-hydroxylauric acid formation (nmol 12-OH formed /60 minutes/mg protein)
Vehicle control [0.1% [v/v] DMSO)	0.040 ± 0.003 (100.0 ± 8.7)	0.396 ± 0.250 (100.0 ± 62.9)	0.314 ± 0.048 (100.0 ± 15.4)
WY 14643 50 µM	0.047 ± 0.011 (119.7 ± 28.7)	1.953 ± 2.604 (492.6 ± 656.9)	0.424 ± 0.087 (135.3 ± 27.7)
WY 14643 100 µM	0.059 ± 0.003 (148.9 ± 7.6)**	3.652 ± 5.775 (921.2 ± 1456.6)	0.328 ± 0.009 (104.6 ± 2.9)
Transfluthrin 3 µM	0.033 ± 0.004 (82.9 ± 10.6)	0.255 ± 0.247 (64.4 ± 62.3)	0.267 ± 0.058 (85.1 ± 18.4)
Transfluthrin 10 µM	0.027 ± 0.003 (68.1 ± 8.5)	4.151 ± 5.585 (1047.1 ± 1408.8)	0.364 ± 0.067 (116.2 ± 21.2)
Transfluthrin 30 µM	0.022 ± 0.004 (56.3 ± 11.0)**	2.510 ± 2.870 (633.1 ± 723.8)	0.278 ± 0.039 (88.6 ± 12.3)
Transfluthrin 100 µM	0.011 ± 0.002 (27.7 ± 4.3)***	0.587 ± 0.661 (148.2 ± 166.8)	0.269 ± 0.033 (85.9 ± 10.6)

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 3 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test; ** statistically different from control $p<0.01$; *** $p<0.001$. Δ For CN^--insensitive acyl CoA oxidation, n = 2 for treatment groups with transfluthrin at 10 µM, 30 µM and 100 µM.

Table 4. Biochemical analysis of BQ, CN^--insensitive acyl CoA oxidation and 12-OH Lauric acid formation in primary female human hepatocytes (donor 1765)

Test Item & Concentration	Benzyloxyquinoline-*O*-debenzylation (nmol 7-hydroxyquinoline formed /minute/mg protein)	CN^--insensitive acyl CoA oxidation (nmol NAD^+ reduced /minute/mg protein)	12-hydroxylauric acid formation (nmol 12-OH formed /60 minutes/mg protein)
Vehicle control (0.1% [v/v] DMSO)	0.090 ± 0.006 (100.0 ± 6.7)	1.050 ± 0.547 (100.0 ± 52.1)	0.420 ± 0.048 (100.0 ± 11.4)
WY 14643 50 µM	0.184 ± 0.031 (204.3 ± 34.1)***	0.303 ± 0.124 (28.9 ± 11.8)*	0.448 ± 0.012 (106.9 ± 2.9)
WY 14643 100 µM	0.269 ± 0.019 (299.5 ± 21.5)***	0.377 ± 0.144 (35.9 ± 13.7)	0.392 ± 0.033 (93.4 ± 7.8)
Transfluthrin 3 µM	0.094 ± 0.008 (104.1 ± 9.1)	0.373 ± 0.029 (35.5 ± 2.7)	0.417 ± 0.020 (99.4 ± 4.8)
Transfluthrin 10 µM	0.081 ± 0.008 (90.3 ± 9.2)	0.534 ± 0.373 (50.8 ± 35.5)	0.453 ± 0.035 (107.9 ± 8.4)
Transfluthrin 30 µM	0.054 ± 0.003 (60.0 ± 3.0)*	0.421 ± 0.291 (40.1 ± 27.7)	0.502 ± 0.010 (119.7 ± 2.5)*
Transfluthrin 100 µM	0.029 ± 0.004 (32.3 ± 4.7)***	0.220 ± 0.123 (21.0 ± 11.7)*	0.514 ± 0.055 (122.5 ± 13.1)*

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 3 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test; * statistically different from control $p<0.05$; ***, $p<0.001$.

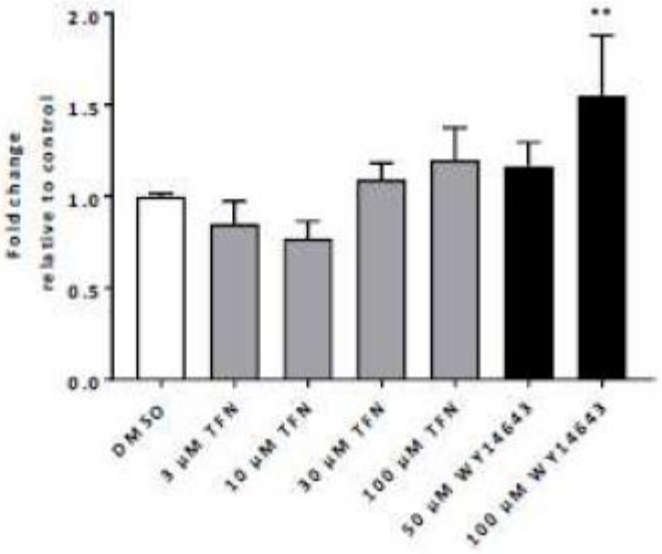
Table 5. Biochemical analysis of BQ, CN^--insensitive acyl CoA oxidation and 12-OH Lauric acid formation in primary female human hepatocytes (donor 1951)

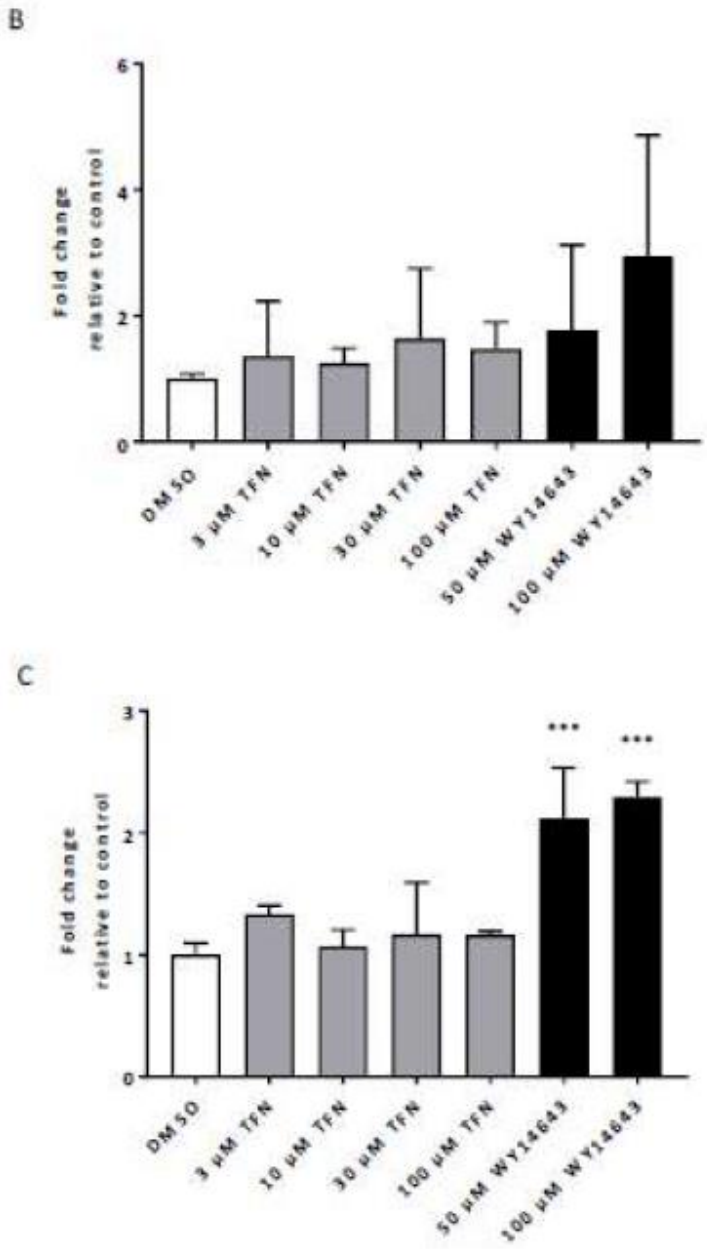
Test Item & Concentration	Benzyloxyquinoline-*O*-debenzylation (nmol 7-hydroxyquinoline formed /minute/mg protein)	CN^--insensitive acyl CoA oxidationΔ (nmol NAD^+ reduced /minute/mg protein)	12-hydroxylauric acid formation (nmol 12-OH formed /60 minutes/mg protein)
Vehicle control (0.1% [v/v] DMSO)	0.081 ± 0.003 (100.0 ± 4.2)	0.272 ± 0.053 (100.0 ± 19.7)	0.398 ± 0.059 (100.0 ± 14.9)
WY 14643 50 µM	0.193 ± 0.021 (238.9 ± 25.6)***	0.977 ± 0.062 (359.6 ± 22.9)	0.465 ± 0.050 (116.7 ± 12.5)
WY 14643 100 µM	0.232 ± 0.012 (287.1 ± 14.6)***	3.623 ± 3.925 (1333.5 ± 1444.5)	0.531 ± 0.049 (133.2 ± 12.2)*
Transfluthrin 3 µM	0.073 ± 0.005 (90.2 ± 5.9)	0.926 ± 0.479 (340.8 ± 176.2)	0.478 ± 0.016 (119.9 ± 4.0)
Transfluthrin 10 µM	0.075 ± 0.006 (92.1 ± 7.5)	0.443 ± 0.295 (163.1 ± 108.6)	0.506 ± 0.046 (127.0 ± 11.5)*
Transfluthrin 30 µM	0.054 ± 0.001 (66.6 ± 1.6)*	0.178 ± 0.060 (65.5 ± 21.9)	0.487 ± 0.053 (122.1 ± 13.3)
Transfluthrin 100 µM	0.037 ± 0.009 (46.2 ± 10.6)***	4.569 ± 5.825 (1681.7 ± 2143.9)	0.415 ± 0.023 (104.3 ± 5.9)

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 3 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test; * statistically different from control $p<0.05$; ***, $p<0.001$. Δ For CN^--insensitive acyl CoA oxidation, n = 2 for treatment groups with transfluthrin at 3 µM, 30 µM, 100 µM and with WY 14643 at 50 µM and 100 µM.

Figure 5. Effect of transfluthrin or WY 14643 on CYP1A1 mRNA expression in primary female human hepatocytes

A

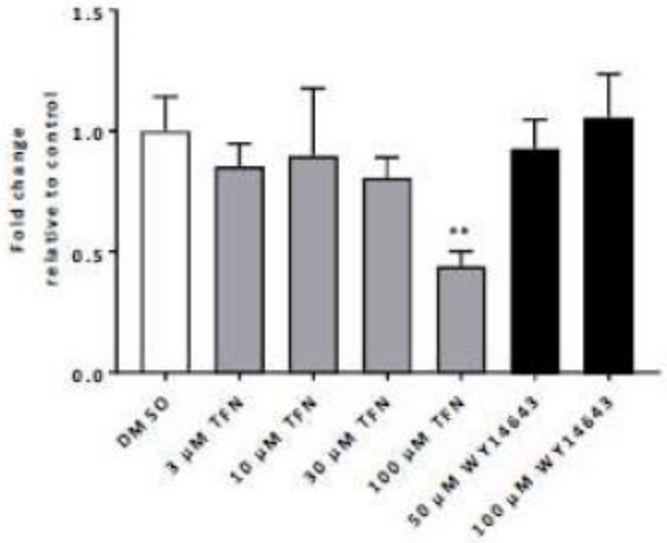




Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; ** statistically different from control $p<0.01$; *** $p<0.001$.

Figure 6. Effect of transfluthrin or WY 14643 on CYP1A2 mRNA expression in primary female human hepatocytes

A



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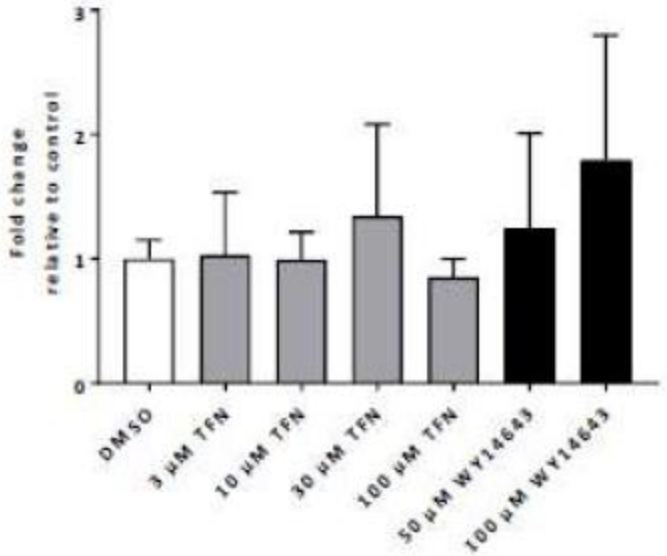
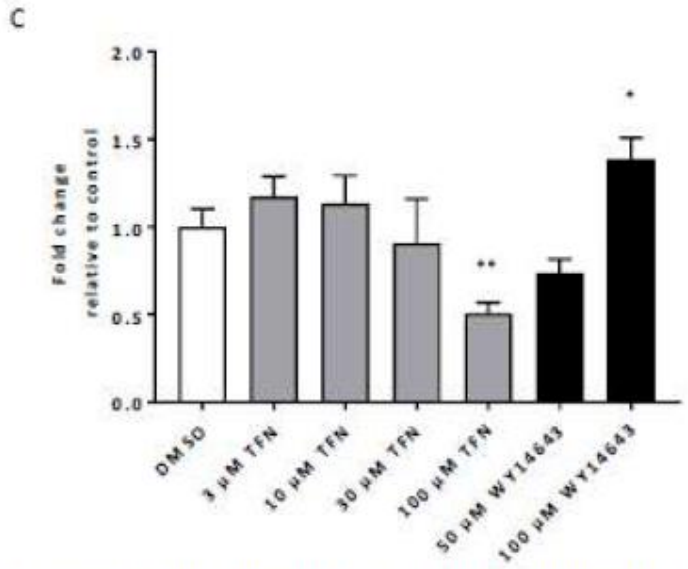
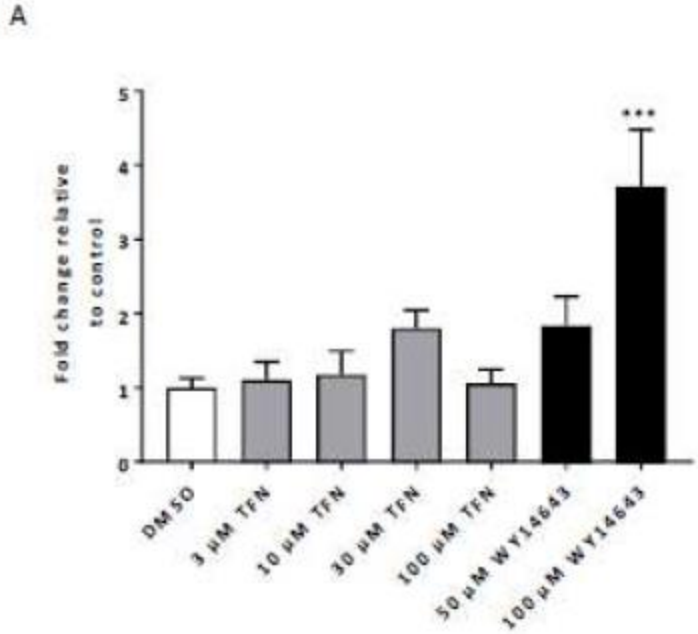




Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$.

Figure 7. Effect of transfluthrin or WY 14643 on CYP2B6 mRNA expression in primary female human hepatocytes



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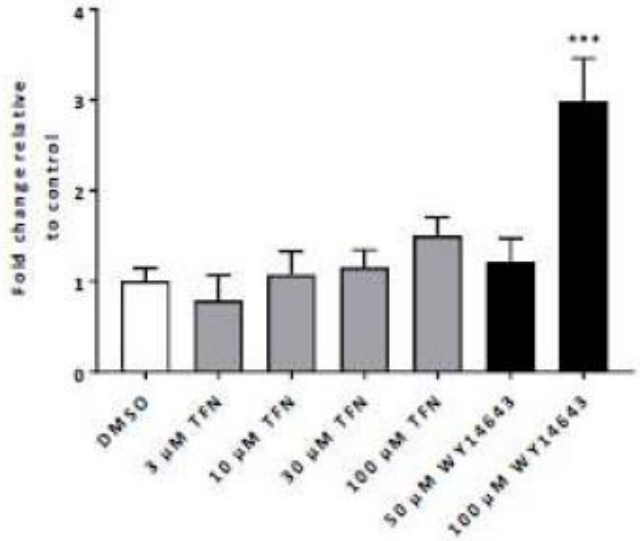

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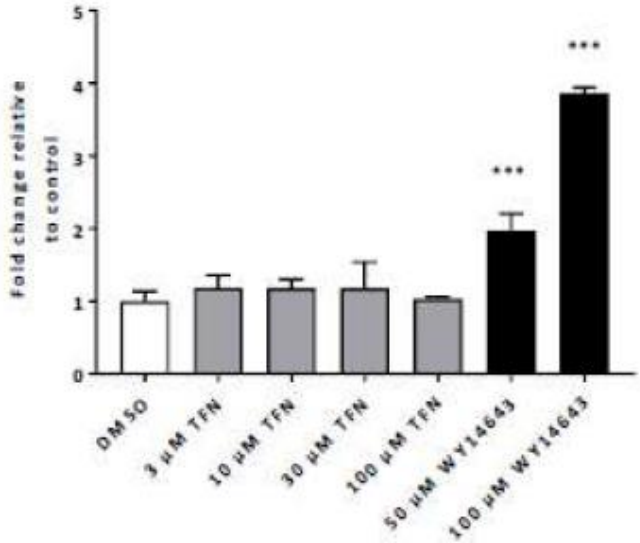
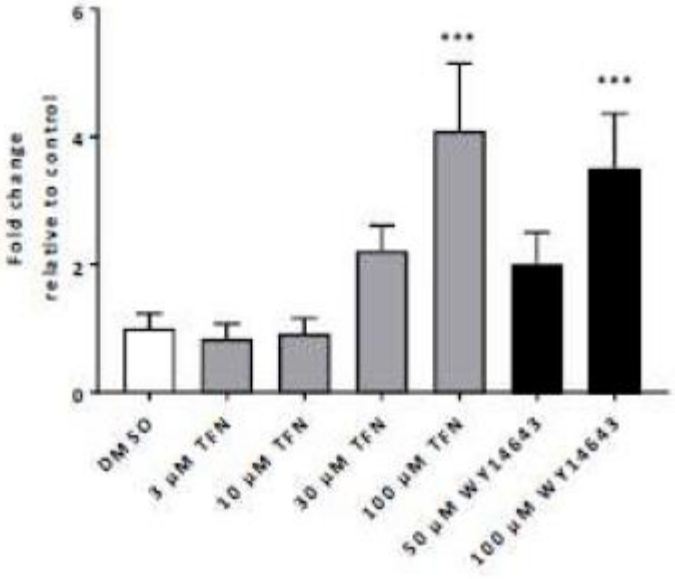


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$.

Figure 8. **Effect of transfluthrin or WY 14643 on CYP3A4 mRNA expression in primary female human hepatocytes**

A



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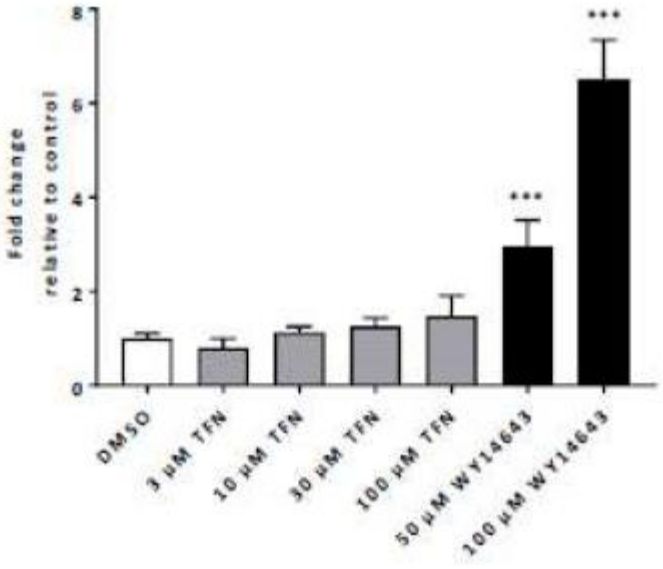
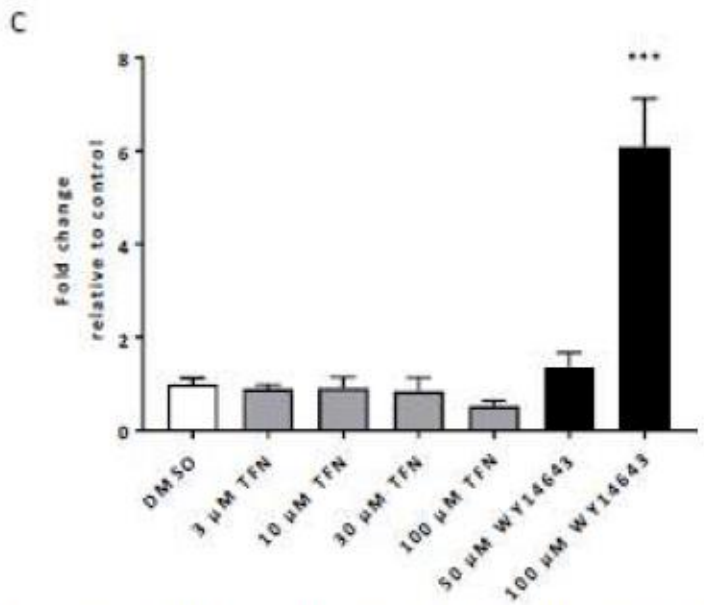
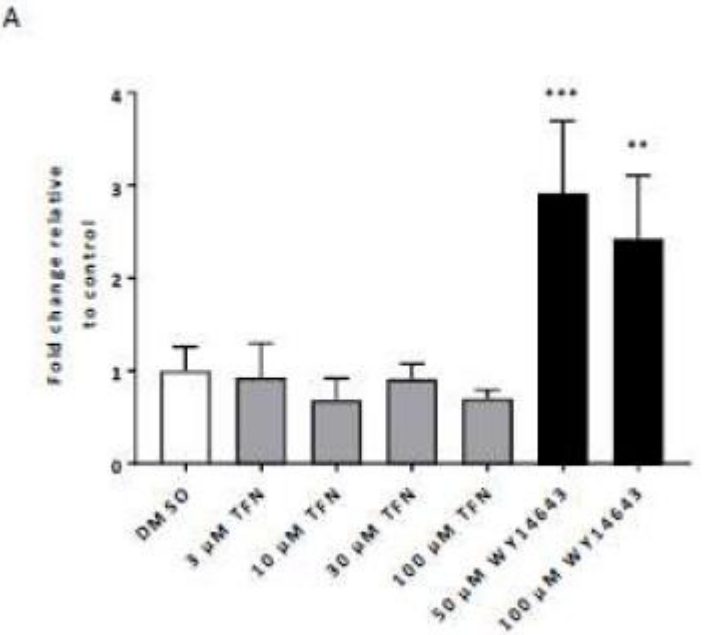




Values are Mean = SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$.

Figure 9: Effect of transfluthrin or WY 14643 on CYP4A11 mRNA expression in primary female human hepatocytes



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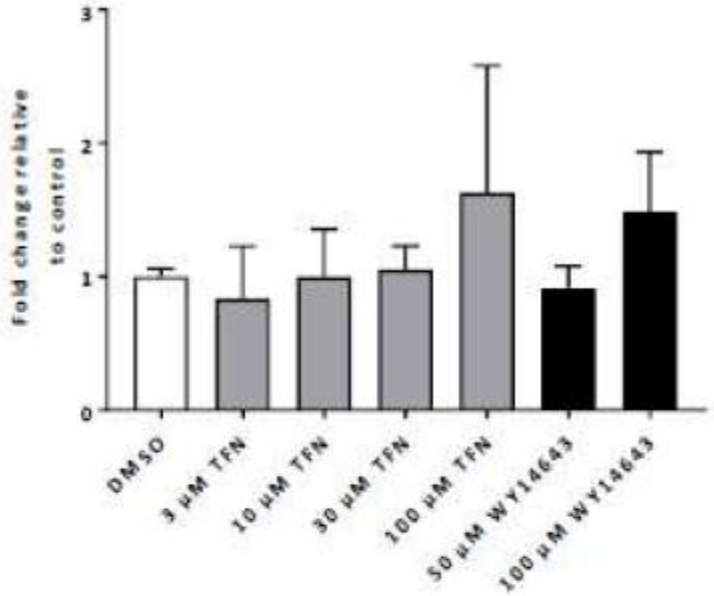

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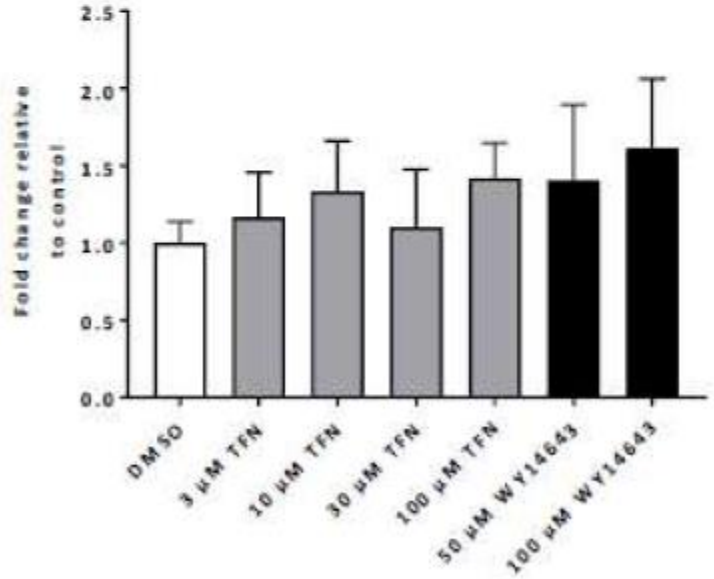
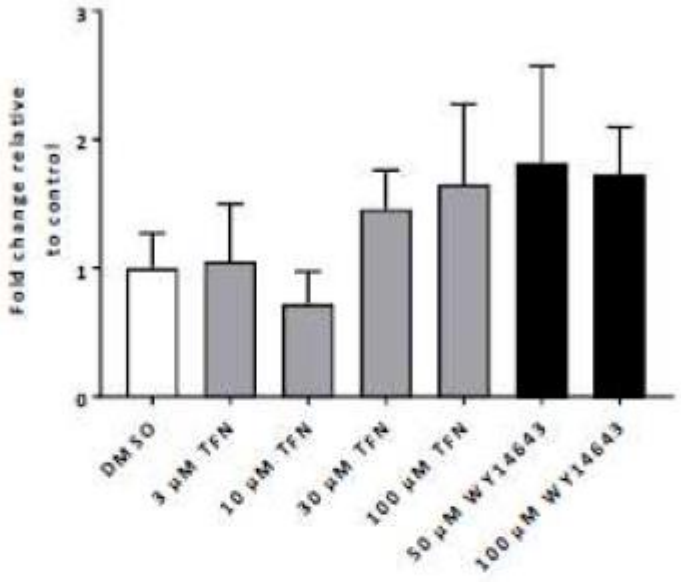


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$.

Figure 10: **Effect of transfluthrin or WY 14643 on ACOX1 mRNA expression in primary female human hepatocytes**

A



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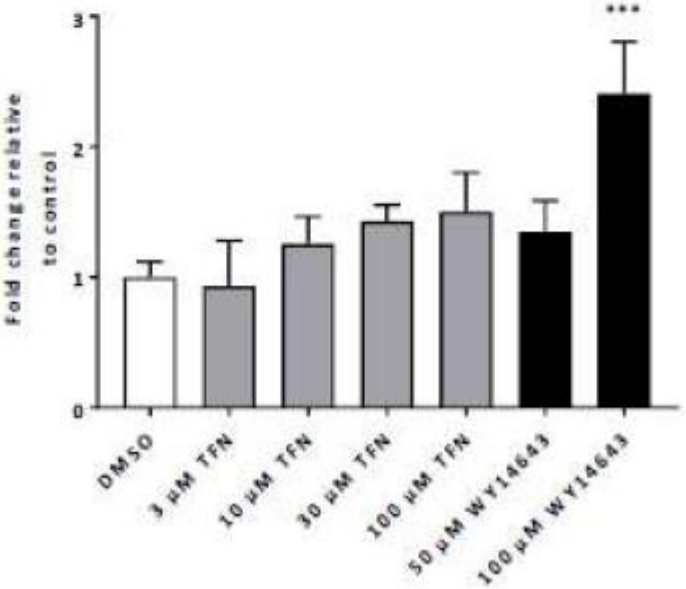

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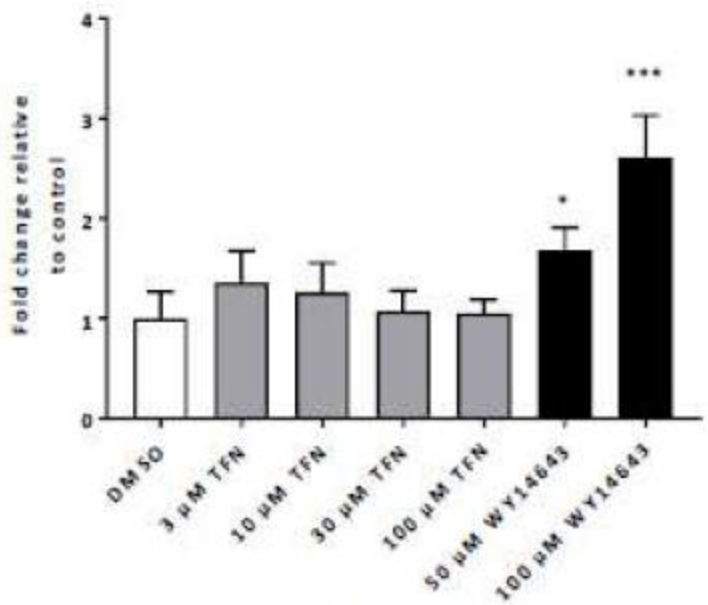


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed using a one way analysis of variance followed by a Dunnett's multiple comparison test; * statistically different from control $p<0.05$; ** $p<0.01$.

Table 6. Taqman® analysis of CYP1A1, CYP1A2, CYP2B6, CYP3A4, CYP4A11 and ACOX1 mRNA in primary female human hepatocytes (donor 314A)

Test Item & Concentration	CYP1A1	CYP1A2	CYP2B6	CYP3A4	ACOX1	CYP4A11
Vehicle control (0.1% [v/v] DMSO)	1.000 ± 0.016	1.000 ± 0.141	1.000 ± 0.130	1.000 ± 0.237	1.000 ± 0.271	1.000 ± 0.260
WY 14643 50 µM	1.165 ± 0.131	0.928 ± 0.119	1.831 ± 0.396	2.008 ± 0.499	1.819 ± 0.755	2.917 ± 0.778***
WY 14643 100 µM	1.548 ± 0.334**	1.057 ± 0.180	3.712 ± 0.764***	3.514 ± 0.853***	1.733 ± 0.364	2.425 ± 0.685**
Transfluthrin 3 µM	0.850 ± 0.125	0.853 ± 0.094	1.108 ± 0.245	0.839 ± 0.238	1.051 ± 0.449	0.928 ± 0.368
Transfluthrin 10 µM	0.770 ± 0.095	0.897 ± 0.280	1.176 ± 0.320	0.918 ± 0.241	0.724 ± 0.251	0.684 ± 0.241
Transfluthrin 30 µM	1.094 ± 0.090	0.806 ± 0.085	1.803 ± 0.245	2.212 ± 0.401	1.460 ± 0.305	0.911 ± 0.167
Transfluthrin 100 µM	1.200 ± 0.176	0.440 ± 0.061**	1.055 ± 0.193	4.094 ± 1.055***	1.654 ± 0.625	0.702 ± 0.091

Table 7. Taqman® analysis of CYP1A1, CYP1A2, CYP2B6, CYP3A4, CYP4A11 and ACOX1 mRNA in primary female human hepatocytes (donor 1765)

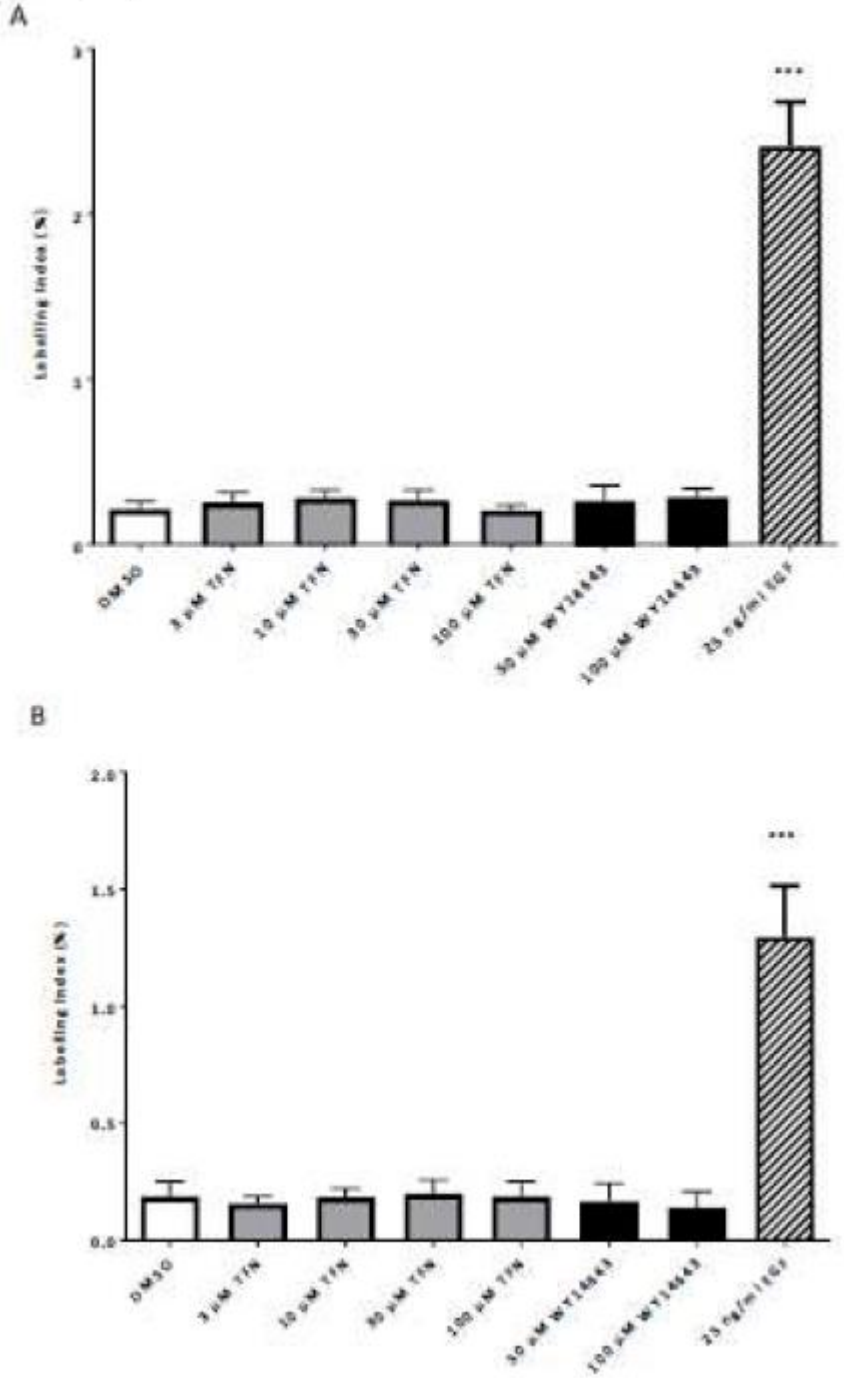
Test Item &| Concentration	CYP1A1	CYP1A2	CYP2B6	CYP3A4	ACOX1	CYP4A11
Vehicle control (0.1% [v/v] DMSO)	1.000 ± 0.081	1.000 ± 0.150	1.000 ± 0.142	1.000 ± 0.103	1.000 ± 0.117	1.000 ± 0.057
WY 14643 50 µM	1.764 ± 1.358	1.247 ± 0.761	1.207 ± 0.266	2.953 ± 0.562***	1.345 ± 0.242	0.908 ± 0.170
WY 14643 100 µM	2.938 ± 1.926	1.796 ± 1.003	2.985 ± 0.477***	6.520 ± 0.824***	2.408 ± 0.396***	1.481 ± 0.452
Transfluthrin 3 µM	1.359 ± 0.871	1.031 ± 0.504	0.786 ± 0.282	0.802 ± 0.196	0.929 ± 0.352	0.834 ± 0.392
Transfluthrin 10 µM	1.251 ± 0.234	0.996 ± 0.221	1.073 ± 0.257	1.131 ± 0.121	1.256 ± 0.209	0.998 ± 0.355
Transfluthrin 30 µM	1.637 ± 1.116	1.344 ± 0.738	1.152 ± 0.193	1.253 ± 0.176	1.428 ± 0.124	1.052 ± 0.176
Transfluthrin 100 µM	1.471 ± 0.435	0.853 ± 0.146	1.502 ± 0.206	1.476 ± 0.433	1.502 ± 0.300	1.629 ± 0.953

Table 8. Taqman® analysis of CYP1A1, CYP1A2, CYP2B6, CYP3A4, CYP4A11 and ACOX1 mRNA in primary female human hepatocytes (donor 1951)

Test Item & Concentration	CYP1A1	CYP1A2	CYP2B6	CYP3A4	ACOX1	CYP4A11
Vehicle control (0.1% (v/v) DMSO)	1.000 ± 0.098	1.000 ± 0.103	1.000 ± 0.135	1.000 ± 0.133	1.000 ± 0.267	1.000 ± 0.137
WY 14643 50 µM	2.116 ± 0.416***	0.737 ± 0.078	1.964 ± 0.240***	1.335 ± 0.343	1.680 ± 0.228*	1.404 ± 0.491
WY 14643 100 µM	2.293 ± 0.127***	1.385 ± 0.121*	3.862 ± 0.081***	6.083 ± 1.044***	2.607 ± 0.422***	1.608 ± 0.452
Transfluthrin 3 µM	1.333 ± 0.072	1.174 ± 0.114	1.185 ± 0.172	0.901 ± 0.078	1.358 ± 0.319	1.165 ± 0.291
Transfluthrin 10 µM	1.068 ± 0.137	1.135 ± 0.158	1.183 ± 0.120	0.911 ± 0.239	1.262 ± 0.294	1.332 ± 0.329
Transfluthrin 30 µM	1.169 ± 0.424	0.908 ± 0.253	1.182 ± 0.357	0.845 ± 0.294	1.074 ± 0.206	1.101 ± 0.375
Transfluthrin 100 µM	1.165 ± 0.028	0.506 ± 0.062**	1.032 ± 0.024	0.524 ± 0.107	1.055 ± 0.135	1.419 ± 0.225

Data expressed as fold change over vehicle control. Values are Mean ± SD; n = 3 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test; ** statistically different from control $p<0.01$; ***, $p<0.001$.

Figure 11: **Effect of transfluthrin, WY 14643 or EGF on replicative DNA synthesis (S-phase) in primary female human hepatocytes**



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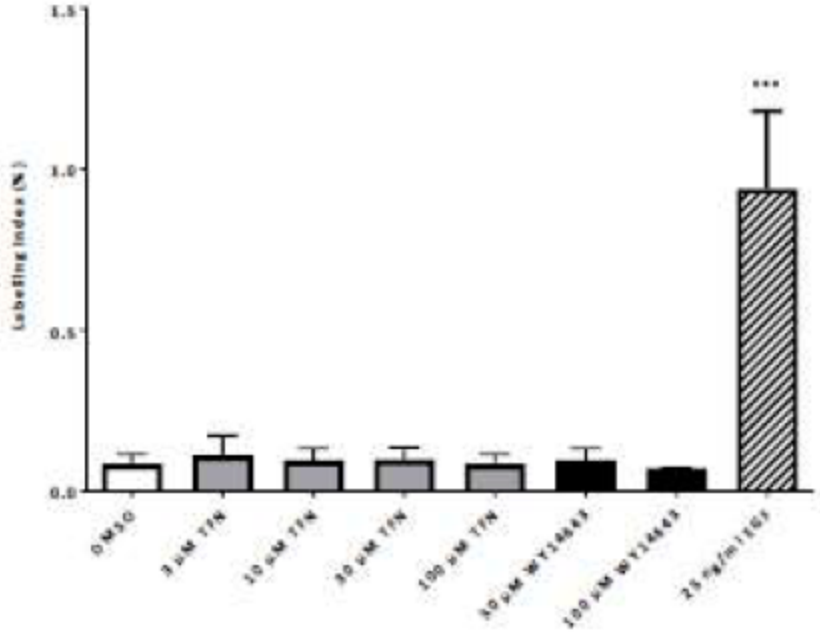


Values are Mean ± SD. n = 3 per group. (A) Donor 314A, (B) Donor 1765, (C) Donor 1951. Statistical analysis was performed between control and treated (excluding EGF) using a one way analysis of variance followed by a Dunnett's multiple comparison test, no statistically significant difference were observed. A Student's t-test (2-sided) was performed on EGF compared to DMSO; *** statistically different from control $p<0.001$.

Table 9: **Effect of transfluthrin, WY 14643 or EGF on replicative DNA synthesis (S-phase) in primary female human hepatocytes**

Test Item & Concentration	S-Phase labelling index		
	Donor 314A	Donor 1765	Donor 1951
Vehicle control (0.1% [v/v] DMSO)	0.212 ± 0.049 (100.0 ± 23.2)	0.084 ± 0.032 (100.0 ± 37.6)	0.186 ± 0.065 (100.0 ± 34.9)
WY 14643 50 µM	0.264 ± 0.090 (124.5 ± 42.7)	0.097 ± 0.036 (114.9 ± 43.2)	0.165 ± 0.076 (89.0 ± 41.0)
WY 14643 100 µM	0.285 ± 0.051 (134.4 ± 24.2)	0.071 ± 0.001 (84.0 ± 1.6)	0.139 ± 0.069 (74.9 ± 37.0)
Transfluthrin 3 µM	0.252 ± 0.062 (119.1 ± 29.3)	0.111 ± 0.061 (131.9 ± 73.0)	0.156 ± 0.032 (83.9 ± 17.2)
Transfluthrin 10 µM	0.278 ± 0.051 (131.2 ± 23.9)	0.096 ± 0.037 (114.0 ±43.5)	0.184 ± 0.038 (98.9 ± 20.5)
Transfluthrin 30 µM	0.266 ± 0.059 (125.9 ± 28.1)	0.097 ± 0.038 (115.8 ± 44.7)	0.199 ± 0.059 (106.9 ± 32.0)
Transfluthrin 100 µM	0.205 ± 0.033 (96.7 ± 15.4)	0.085 ± 0.031 (100.6 ± 36.7)	0.186 ± 0.065 (100.2 ± 34.8)
EGF$^{\Delta}$ 25 ng/ml	2.412 ± 0.265 (1139.9 ± 125.3)***	1.005 ± 0.219 (1194.4 ± 260.4)***	1.296 ± 0.220 (697.3 ± 118.4)***

Values are Mean ± SD. Values in parenthesis are mean % control ± SD; n = 5 per group. A one way-ANOVA was performed on the results followed by Dunnett's multiple comparisons test. Δ An unpaired t-test (two-tailed) was performed on the results for EGF treated hepatocytes; *** statistically different from control $p<0.001$.

3.10 Reproductive toxicity

3.10.1 Animal data

3.10.1.1 Study 1 – Multigeneration Reproduction Oral Toxicity Study in Rats

Doc. IIIA/ Section A6.8.2 BPD Data set IIA/ Annex Point IIA6.8.2	**Multigeneration Reproduction Oral Toxicity Study in Rats**	
	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
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).*	
GLP	*Yes*	
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.* MATERIALS AND MethodS	
Test material	As given in section 2, NAK 4455 (current name transfluthrin, suggested name benfluthrin in report)	
Lot/Batch number	*Batch no. 250987*	
Specification	*As given in Section 2 of Doc IIIA.*	
Description	*brown solid.*	
Purity	*94.4 – 95.3%*	
Stability	*Analyzed 4 times, listed as stable for 3 weeks in the diet.*	

Test Animals	
Species	*Rat*
Strain	*Wistar/HAN (Kfm: WIST, outbred, SPF quality).*
Source	*KFM, Kleintierfarm Madoerin AG, CH 4414 Fuellinsdorf / Switzerland*
Sex	*Male & Female*
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.*
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.*
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.*
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.*
Control animals	Yes
Administration/ Exposure	
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.*
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.
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
Type	In food, mixed into a microgranulated diet and pelleted.
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.
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.
Concentration in vehicle	a.s. concentration in acetone not relevant for final concentration in food: 20, 200, 1000 ppm
Total volume applied	Not applicable
Controls	Plain diet, granulated diet processed into pellets similarly to test article diet.

Examinations	
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.
Body weight	The body weight and weight gain of all animals was monitored. Mean body weight of groups was recorded.
Food/water consumption	Food consumption was monitored in all animals, but water intake was not.
Oestrus cycle	No
Sperm parameters	No
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
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.
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.
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.
Further remarks	Brain and thymus were not weighed a suggested in current guidance. No treatment-related effects were noted in any tissue weighed. Results and Discussion

Effects

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
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
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
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.*	
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.*	
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.*	

Doc. IIIA/ Section A6.8.2 BPD Data set IIA/ Annex Point IIA6.8.2	Multigeneration Reproduction Oral Toxicity Study in Rats	
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).	X
	Clinical biochemistry, except for slight increase in liver triglycerides of F1 parent animals at 1000 ppm.	
	Organ weights of parent animals, except for the P generation at 1000 ppm. Macroscopic examination of all animals, and microscopic examination of F2B pups.	
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.*	X
	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.	
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.*	
LO(A)EL		
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
Parent females	*1000 ppm - increased kidney weights and cellular abnormalities*	X
F1 males	1000 ppm - slight decrease in liver triglycerides.	X
F1 females	1000 ppm - based on slightly decreased body weight gain in F1 females, and slight decrease in liver triglycerides.	X
F2 males	No effects noted at any dose.	
F2 females	No effects noted at any dose.	
NO(A)EL		
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
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

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
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
F2 males	1000 ppm (range 45-191 mg/kg bw/day) for growth.	
F2 females	1000 ppm (range 45-191 mg/kg bw/day) for growth.	
Reliability	*2*	

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.	

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	

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

		control		20 ppm		200 ppm		1000 ppm	
Parameter	Genera-tion	m	f	m	f	m	f	m	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 Liver Spleen Testes/Ovaries	**P**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**Inc.** **Inc.** **-** **-**	**Inc** **-** **-** **-**
	Kidney Liver Spleen Testes/Ovaries	**F_1**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**	**-** **-** **-** **-**

Continued

Reproductive Performance										
Mean precoital time		**P, F1A F1B**	**3.9 2.9**		**2.9 3.1**		**2.5 2.8**		**2.5 2.5**	
		F_1, F2A F2B	**3.5 2.3**		**2.8 2.8**		**4.0 4.6**		**3.6 3.5**	
Percentage mating		**P, F1A F1B**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**
		F_1, F2A F2B	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**	**100 100**
Fertility index (%)	*(same as conception rate)*	**P, F1A F1B**	**100 100**		**90 100**		**100 100**		**96.6 86.2**	
		F_1, F2A F2B	**80.8 76.9**		**92.3 100**		**92.3 88.5**		**92.3 84.6**	
Gestation index (%)		**P, F1A F1B**		**100 100**		**100 100**		**100 100**		**100 100**
		F_1, F2A F2B		**100 95.0**		**100 96.2**		**100 95.7**		**100 100**

	Number of females mated/pregnant /rearing pups as used for litter data	P generation**, F1A** **F1B**	**30/30/30** **29/29/29**	**30/27/27** **30/30/30**	**30/30/30** **30/30/30**	**29/28/26** **29/25/25**
		F_1 generation**, F2A** F2B	**26/21/21** **26/20/19**	**26/24/24** **26/26/25**	**26/24/24** **26/23/22**	**26/24/24** **26/22/22**

Continued

Litter size, pup survival of dams rearing pups (litters were culled to 8 pups on day 4)	Mean, living at first check	P**, F1A** **F1B**	**11.6** **11.6**	**10.9** **10.0**	**10.9** **10.9**	**11.5** **11.8**
		F_1**, F2A** **F2B**	**10.0** **9.6**	**9.5** **10.3**	**11.0** **10.7**	**12.3** **10.7**
	Mean, dead at first check	P**, F1A** **F1B**	**0.13** **0.07**	**0.15** **0.13**	**0.13** **0.03**	**0.08** **0.0**
		F_1**, F2A** **F2B**	**0.05** **0.05**	**0.21** **0.28**	**0.29** **0.23**	**0.21** **0.23**
	% postnatal loss days 0-4	P**, F1A** **F1B**	**0.9** **0.3**	**1.7** **2.3**	**1.8** **0.6**	**1.0** **0.3**
		F_1**, F2A** **F2B**	**1.9** **0.0**	**3.0** **1.2**	**1.5** **0.4**	**1.0** **2.5**
	% postnatal loss days 4-21	P**, F1A** **F1B**	**1.3** **0.4**	**0.5** **1.8**	**1.8** **0.9**	**1.5** **0.5**
		F_1**, F2A** **F2B**	**2.6** **0.0**	**2.3** **1.6**	**0.6** **0.6**	**1.0** **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** **F1B**	**0.13** **0.07**	**0.15** **0.13**	**0.13** **0.03**	**0.08** **0.0**
		F_1, F2A **F2B**	**0.05** **0.05**	**0.21** **0.28**	**0.29** **0.23**	**0.21** **0.23**

Continued

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

			control		20 ppm		200 ppm		1000 ppm	
Parameter		Generation	m	f	m	f	m	f	m	f
Mortality	Overall	**F_1 A** **F_1 B**	**3** **-**	**3** **2**	**3** **5**	**3** **6**	**3** **3**	**7** **1**	**2** **1**	**4** **1**
		F_2 A **F_2 B**	**3** **-**	**5** **-**	**6** **3**	**5** **3**	**3** **1**	**2** **1**	**3** **4**	**2** **3**
	First day	**F_1 A** **F_1 B**	**-** **-**	**-** **1**	**-** **1**	**-** **4**	**1** **-**	**2** **-**	**1** **-**	**-** **-**
		F_2 A **F_2 B**	**-** **-**	**1** **-**	**3** **-**	**1** **1**	**-** **-**	**-** **-**	**-** **-**	**-** **1**
Sex Ratios, % male	*(After parturition*	**F_1 A** **F_1 B**	**50.1** **50.4**		**50.0** **56.8**		**50.8** **48.3**		**47.0** **55.6**	

	)	**F_2 A** **F_2 B**	**48.3** **54.4**	**50.6** **48.6**	**52.1** **53.4**	**49.8** **50.8**
Organ Weights		**There were no test article related changes in the organ weights of any of the pups.**				

3.10.1.2 Study 2 - Teratogenicity Study - Rat

Doc. IIIA/ Section A6.8.1/01 **Teratogenicity Study - Rat**
BPD Data set IIA/ Annex Point VI.6.8.1/01

			Official use only
		Reference	
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	
2.1	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).	
2.2	GLP	*Yes*	
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.	
		3 MATERIALS AND MethodS	
Test material		NAK 4455 (transfluthrin)	
3.1.1	Lot/Batch number	*Batch No. 130187*	
	Specification	*As given in Section 2 of Doc IIIA.*	
Description		*Brown liquid.*	
Purity		*94.5%*	
Stability		*Emulsions of the test article up to 5% concentration within aqueous Emulphor vehicle remained stable for at least 4 weeks.*	
Test Animals			
3.2.1	Species	Rat	
	Strain	Charles River Crl:CD BR strain	
Source		Charles River Breeding Laboratories, Portage, Michigan, U.S.A.	

Doc. IIIA/ Section A6.8.1/01 **BPD Data set IIA/ Annex Point VI.6.8.1/01**	**Teratogenicity Study - Rat**	
Sex	Female and Male	
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.	
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.*	
Control animals	Yes	
Mating period	Until evidence of copulation was found (sperm observed in vaginal smear) for each individual female.	
Administration/ Exposure	*Oral, by gavage.*	
3.3.1 Duration of exposure	*Days 6 – 15 post-insemination, for a total of 10 consecutive doses.*	
Post exposure period	4 days (rats sacrificed at presumed day 20 of pregnancy).	
	Oral	
Type	Gavage	
Dose levels	0 (Control), 25, 55 or 120 mg kg bw/day. Doses were chosen based on a range finding study.	X 125
Vehicle	5% Emulphor EL-719 (polyethoxylated vegetable oil), 95% distilled water	
Concentration in vehicle	0, 2.5, 5.5 or 12.5 mg/ml emulsion in 5% aqueous Emulphor.	
Total volume applied	10 ml/kg	
Controls	Vehicle, volume 10 ml/kg.	
Examinations		
3.4.1 Body weight	Yes, days 0, 6, 8, 10, 12, 15, and 20 during gestation.	
Food consumption	Yes, days 1, 6, 7, 12, 16, and 20 during gestation.	
Clinical signs	Dams were observed daily. General appearance and behaviour, body weight and food consumption.	
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.	
Other maternal observations	Gross pathology at necropsy, including some organ weights (uterus, placenta, liver).	
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 Bouin'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.	
General	Dam reproductive efficiency, fetal and placental weights, viability and sex ratios, and incidence of malformations/variations.	
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.
Soft tissue		Yes, approximately 50% of foetuses were examined both externally and stereoscopically (viscera).
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 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.
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.
		4 Results and Discussion
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.*
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.*
4.3	Other effects	*None noted*
		5 Applicant's Summary and conclusion
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 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.*
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.*
5.3	Conclusion	
5.3.1	LO(A)EL maternal toxic effects	*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.*
5.3.2	NO(A)EL maternal toxic effects	*25 mg/kg bw/day.*
5.3.3	LO(A)EL embryotoxic /	*Greater than 125 mg/kg bw/day, based on the absence of findings at the highest dose tested.*

	teratogenic effects	
5.3.4	NO(A)EL embryotoxic / teratogenic effects	*125 mg/kg bw/day.*
5.3.5	Reliability	*1*
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	
	Evaluation by Rapporteur Member State
Date	*1-3-2007*
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.*
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.*
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.*
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 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)									

3.10.1.3 Study 3 – Teratogenicity Study – Rabbit

Doc. IIIA/ Section A6.8.1/02 **BPD Data set IIA/ Annex Point VI.6.8.1**	**Teratogenicity Study – Rabbit** **(translated from German original)**

			Official use only
		Reference	
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	
2.1	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).
2.2 GLP	*Yes*
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.
	3 MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
3.1.1 Lot/Batch number	*Mixed batch 250 987*
Specification	*As given in Section 2 of Doc IIIA.*
Description	*Brown-yellow clear liquid.*
Purity	*94.5% (27 Oct 1987), 95% (27 April 1988 retest)*
Stability	*Not specified other than that tests were made for stability and homogeneity in the administered formulation until 27 October 1988.*
Test Animals	
3.2.1 Species	Rabbit
Strain	CHBB:Himalayan strain
Source	Thomae Co., Biberach a.d. Riss
Sex	Female and Male
Age/weight at study initiation	Males were sexually mature and >2500 g. Females were sexually mature, nulliparous and 2231-3219 g.
Number of animals per group	*15 females and males/group; current guidelines suggest 20 females.*
Control animals	Yes, concurrent and historical controls.
Mating period	Mating was observed with one male and one female per cage. This was defined as day zero of gestation.
Administration/ Exposure	
3.3.1 Duration of exposure	*Days 6 – 18 post-insemination, for a total of 13 consecutive doses.*
Post exposure period	10 days (rabbits sacrificed at presumed day 29 of pregnancy).
	Oral
Type	Gavage
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.
Vehicle	5% Cremophor EL emulsion (glycerine-polyethylene glycol ricinoleate; non-ionic solubilizer made with ethylene oxide and castor oil), 95% distilled water
Concentration in vehicle	0, 0.3, 1.0 or 3.0% .
Total volume applied	5 ml/kg bw
Controls	Vehicle, volume 5 ml/kg bw

Doc. IIIA/ Section A6.8.1/02 BPD Data set IIA/ Annex Point VI.6.8.1	Teratogenicity Study – Rabbit (translated from German original)	
Examinations		
3.4.1 Body weight	Day 0, then daily during dosing (days 6-18) and at day 29. Weight gain during gestation was documented.	
Food consumption	Monitored but data not given (reduced food intake noted in observations for some dams)	
Clinical signs	Dams were observed daily for appearance and behaviour.	
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
Other maternal observations	Gross pathology at necropsy. One high-dose and one midrange dose dam died during treatment.	
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.	
General	Dam reproductive efficiency.	
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.	
Soft tissue	Yes, all foetuses were examined externally and organs examined visually.	
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
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%.	
	4 Results and Discussion	
4.1 Maternal toxic Effects	*Tremor occurred after dosing in one mid-dose and one high-dose dam, followed by death. The central nervous system symptoms seen in these 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.*	
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.	
4.3	Other effects	*None noted*	
		5 Applicant's Summary and conclusion	
5.1	Materials and 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.*	
5.2	Results and 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.*	
5.3	Conclusion		
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.*	
5.3.2	NO(A)EL maternal toxic effects	*15 mg/kg bw/day.*	

Doc. IIIA/ Section A6.8.1/02 BPD Data set IIA/ Annex Point VI.6.8.1		Teratogenicity Study – Rabbit (translated from German original)
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.*
5.3.4	NO(A)EL embryotoxic / teratogenic effects	*150 mg/kg bw/day.*
5.3.5	Reliability	*1*
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.*

Evaluation by Competent Authorities	
	Evaluation by Rapporteur Member State
Date	*01-03-2007*
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).*
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.*
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.*
Reliability	*2*
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.*
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 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.					

3.10.1.4 Study 4 – Developmental neurotoxicity Study

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

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*	

Doc. IIIA/ Section A 6.9 BPD Data set IIA/ Annex Point VI.6.9	**Neurotoxicity** *Developmental neurotoxicity Study*	
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*	
3.2 Test Animals		
3.2.1 Species	*Rat*	
3.2.2 Strain	*Wistar crl:WI(Han)*	
3.2.3 Source	*Charles River Laboratories, Inc., Raleigh, NC*	
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		
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.	
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.	
5.1 Materials and methods	5. Applicant's Summary and conclusion 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. Treatment-related effects attributed to exposure to transfluthrin were as follows: **Maternal** 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. **Offspring** 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.	X
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

3.10.2 Human data

No data available.

3.10.3 Other data (e.g. studies on mechanism of action)

No data available.

3.11 Specific target organ toxicity – single exposure

3.11.1 Animal data

Please also refer to section 3.1.

3.11.1.1 Study 1 – Acute Oral Rat neurotoxicity Study

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

1 Reference

Official use only

Doc. IIIA/ Section A 6.9	**Neurotoxicity**
BPD Data set IIA/ Annex Point VI.6.9	*Acute Oral Rat neurotoxicity Study*
Data protection	Yes
Data owner	Bayer CropScience
Companies with letters of access	
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
	Guidelines and Quality Assurance
Guideline study	*This study addresses only the motor activity (open field study) part of OECD TG 424.*
GLP	*Yes*
Deviations	*Yes; neurohistology, clinical observations, and a complete functional observational battery are not included.*
	MATERIALS AND MethodS
Test material	*BAY U 4619 (transfluthrin)*
Lot/Batch number	*816679301*
Specification	
Description	*Yellowish crystalline compound*
Purity	*95.5%*
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.*
Test Animals	
Species	*Rat*
Strain	*Wistar (HsdCpb: WU)*
Source	*Harlan Winklemann GmbH, Gartenstrasse 27, D-33178 Borchen, FRG*
Sex	*Male*
Age/weight at study initiation	*Approximately 6 weeks of age, within a weight range of 173-223 g.*
Number of animals per group	6 – for combined temperature/catalepsy test *10 – for open field test of psychomotoric activity*
Control animals	*Yes – concurrent control group given vehicle only*
Administration/ Exposure	
Duration of treatment	*Single dose*
Frequency of exposure	*1 dose*
Postexposure period	*Up to 4 hours*
Oral	
Type	*Gavage*
Concentration	*0, 10, 30, 100 mg/kg in a volume of 5 mL/kg*
Vehicle	*PEG 400*
Controls	*PEG 400*
Examinations	
Observations	
Clinical signs	*No*
Mortality	*No*
Body weight	*No*
Food consumption	*No*
Water consumption	*No*
Ophthalmoscopic examination	*No*

Haematology	*No*
Clinical Chemisty	*No*
Urinalysis	*No*
Sacrifice and pathology	
Organ Weights	*No*
Gross and histopathology	*No*
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.*
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.*
Further remarks	Results and Discussion
Observations	
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.*
Mortality	*None.*
Body weight/body weight gain	*Not given.*
Food consumption and compound intake	*Not given.*
Functional Observational Battery (FOB)	*Not noted*
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.
Sacrifice and pathology	
Organ weights	*Not measured*
Neuro-histopathology	*Not evaluated*
Other	*None*
	Applicant's Summary and conclusion
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.*
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 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.*
Conclusion	
LO(A)EL	*No treatment-related effect was seen in this study.*
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.*
Other	*Treatment with transfluthrin is not considered to influence the acute motor activity of rats.*
Reliability	*2. This study adequately reports motor activity during the initial 4 hours post-dosing, so partially fulfils OECD 424 guidelines*
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*
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	

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				

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				

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				

3.11.2 Human data

No data available.

3.11.3 Other data

No data available.

3.12 Specific target organ toxicity – repeated exposure

3.12.1 Animal data

3.12.1.1 Study 1 – 28-Day oral rat study

Doc. IIIA/ Section A6.3.1	**Short-term repeated dose toxicity**
BPD Data set IIA/ Annex Point VI.6.3	28-Day oral rat study

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD 407 Repeated Dose Oral Toxicity Rodent: 28-day or 14-day Study (1981)	
GLP	Yes	
Deviations	No	

	MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
Lot/Batch number	130187
Specification	As given in section 2
Description	Liquid from 50C (solid below 50C), dark brown
Purity	95.0%
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.
Test Animals	
Species	Rat
Strain	Bor:WISW (SPF-Cpb) (Wistar)
Source	Versuchstierzucht Winkelmann, Borchen
Sex	Male and female
Age/weight at study initiation	7 – 8 weeks Mean weight at start of 153 g (males) and 136 g (females)
Number of animals per group	30 rats/sex/group (except high dose group which had 35/sex/group)
Control animals	Yes
Administration/ Exposure	Oral (gavage)
Duration of treatment	28 days
Frequency of exposure	Daily
Observation period	4 weeks
Oral	
Type	Gavage
Concentration	0, 10, 50 250 mg/kg bw
Vehicle	Polyethylene glycol E 400
Concentration in vehicle	0. 0.2, 1.0, 5.0 %
Total volume applied	5 mL/kg bw
Controls	Vehicle
Examinations	
Observations	
Clinical signs	Observed daily.
Mortality	Observed daily.
Body weight	Weekly.
Food consumption	Calculated weekly.
Water consumption	No
Ophthalmoscopic examination	No
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.
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.
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
Sacrifice and pathology	
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
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.
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.
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.
Further remarks	None
	Results and Discussion
Observations	
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).*
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).
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.
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).
Ophthalmoscopic examination	Not applicable
Blood analysis	
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.
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.
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.
Sacrifice and pathology	
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.
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.
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.
	Applicant's Summary and conclusion
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.
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.
Conclusion	
LO(A)EL	LOAEL was 250 mg/kg bw based on tremor (and death) in both sexes.
NO(A)EL	NOAEL was 50 mg/kg bw in both sexes.
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.
Reliability	1
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

3.12.1.2 Study 2 – 21-Day dermal rabbit study

Doc. IIIA/ Section A6.3.2 **Repeated dose toxicity**
BPD Data set IIA/ Annex Point VI.6.3 21-Day dermal rabbit study

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		

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 Guidelines and Quality Assurance
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)
GLP	Yes
Deviations	No MATERIALS AND MethodS
Test material	NAK 4455 techn. (transfluthrin)
Lot/Batch number	250987
Specification	As given in section 2
Description	Liquid from 50C (solid below 50C), dark brown
Purity	95.0%
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.
Test Animals	
Species	Rabbit
Strain	HC:NZW (New Zealand White)
Source	Interfauna UK Limited, England
Sex	Male and female
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)
Number of animals per group	5 rabbits/sex/group (except control and high dose group which had 10/sex/group)
Control animals	Yes
Administration/ Exposure	Dermal
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

Duration of treatment	21 days
Frequency of exposure	5 days per week
Observation period	14 days
Dermal	
Area covered	>10 % of body surface
Occlusion	Semiocclusive
Vehicle	Cremophor EL (2% v/v) in physiological saline
Concentration in vehicle	0, 1, 10, 50 %
Total volume applied	2 mL/kg bw
Duration of exposure	6 h
Removal of test substance	After exposure, treatment area cleaned with soap and water.
Controls	Vehicle

Examinations	
Observations	
Clinical signs	Yes, observed daily.
Mortality	Yes, observed daily.
Body weight	Yes, weekly.
Food consumption	Yes, calculated weekly.
Water consumption	No
Ophthalmoscopic examination	No
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.
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.
Urinalysis	Not performed
Sacrifice and pathology	
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
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.
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.
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.
Further remarks	
	Results and Discussion

Observations	
Clinical signs	No effects
Mortality	No mortalities at any dose
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).
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.
Ophthalmoscopic examination	Not applicable
Blood analysis	
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.
Clinical chemistry	No effects
Urinalysis	Not applicable
Sacrifice and pathology	
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.
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).
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.
	Applicant's Summary and conclusion
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 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.
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.
Conclusion	
LO(A)EL	No systemic effects were seen, thus no LOAEL was established.
NO(A)EL	NOAEL (systemic) was 1000 mg/kg bw in both sexes.
Other	
Reliability	1
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

3.12.1.3 Study 3 – 18-Week oral rat study

Doc IIIA/Section A 6.4.1/01 **BPD Data set IIA/ Annex Point VI. 6.4**	**Subchronic toxicity** 18-Week oral rat study

Official use only

	Reference
Data protection	Yes
Data owner	Bayer CropScience
Companies with letters of access	
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
	Guidelines and Quality Assurance
Guideline study	Yes
	US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)
GLP	Yes
Deviations	No
	MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
Lot/Batch number	130187
Specification	As given in section 2
Description	Liquid from 50ºC (solid below 50ºC), dark brown
Purity	95.0%
Stability	Verified by analysis during the study.
Test Animals	
Species	Rat
Strain	Bor:WISW (SPF-Cpb) (Wistar)
Source	Winkelmann, Borchen
Sex	Male and female
Age/weight at study initiation	6 weeks Weight range at start of study 95-116 g (males) and 89-113 g (females)
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")
Control animals	Yes
Administration/ Exposure	Oral (diet)
Duration of treatment	95-97 days (plus a 4-week exposure for satellite groups, for duration of 126-127 days)
Frequency of exposure	Continuous (diet, *ad-lib*)
Postexposure period	None
Oral	
Type	In food
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
Vehicle	Test compound mixed with 1% peanut oil then food powder
Concentration in vehicle	95%
Total volume applied	Not applicable
Controls	Diet with 1% peanut oil
Examinations	
Observations	
Clinical signs	Yes, observed twice daily.
Mortality	Yes, observed twice daily.
Body weight	Yes, weekly.
Food consumption	Yes, calculated weekly.
Water consumption	Yes, calculated weekly.
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.
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.
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).
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
Sacrifice and pathology	
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.
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.
Other examinations	Enzyme induction: At sacrifice (13 weeks or 19 weeks for satellite groups) animals were examined for enzyme induction in the liver, specifically: N-demethylase, O-demethylase, cytochrome P450 and carnitine acyl transferase.
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.
Further remarks	
	Results and Discussion
Observations	
Clinical signs	No effects
Mortality	No treatment related mortalities were seen.
Body weight gain	No effects
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.
Ophthalmoscopic examination	No effects
Blood analysis	
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.
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.
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 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.
Sacrifice and pathology	
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.
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.
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).
	Applicant's Summary and conclusion
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.	
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). 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.	
Conclusion		
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
NO(A)EL	The no observable adverse effect level is 500 ppm (42 mg/kg bw/day).	X

Other	
Reliability	1
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,* = $p < 0.05$; ** = $p < 0.01$

3.12.1.4 Study 4 – 3-month oral dog study

Doc IIIA/Section A 6.4.1/02	**Repeated dose toxicity** 3-month oral dog study
BPD Data set IIA/ Annex Point VI. 6.4	

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD Guidelines 409 (1981)	
	US EPA FIFRA § 82-1 Subchronic Oral Toxicity (1984)	
GLP	Yes	
Deviations	No	
	MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	250987	
Specification	As given in section 2	
Description	Solid	
Purity	94.5%	
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.	
Test Animals		
Species	Dog	
Strain	Beagle	
Source	Laboratory Research Enterprises, Inc. Michigan, USA	
Sex	Male and female	
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)	
Number of animals per group	4 dogs/sex/group	
Control animals	Yes	
Administration/ Exposure	Oral (dietary)	
Duration of treatment	97 days	
Frequency of exposure	Daily	
Postexposure period	None	
Oral		

Type	In food
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)
Vehicle	Test compound mixed with peanut oil (for dust control) then food powder
Concentration in vehicle	95%
Total volume applied	Not applicable
Controls	Diet
Examinations	
Observations	
Clinical signs	Yes, observed twice daily.
Mortality	Yes, observed twice daily.
Body weight	Yes, weekly.
Food consumption	Yes, recorded daily.
Water consumption	Yes, calculated weekly.
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).
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.
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.
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
Sacrifice and pathology	
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.
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.
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.
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.
Further remarks	
	Results and Discussion
Observations	
Clinical signs	No effects
Mortality	No treatment related mortalities were seen. One female in the control group became moribund due to severe bronchopneumonia and was sacrificed.
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.
Food consumption and compound intake	No treatment related effects were seen on food consumption or compound intake.
Ophthalmoscopic examination	No effects
Blood analysis	
Haematology	There were no treatment related effects.
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.
Urinalysis	No treatment related effects were seen.
Sacrifice and pathology	
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.

Doc IIIA/Section A 6.4.1/02 BPD Data set IIA/ Annex Point VI. 6.4	**Repeated dose toxicity** 3-month oral dog study
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.
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. Applicant's Summary and conclusion
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).
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
Conclusion	
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
NO(A)EL	The no observable adverse effect level is 350 ppm (14 mg/kg bw/day).
Other	
Reliability	1
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

Doc IIIA/Section A 6.4.1/02	**Repeated dose toxicity** 3-month oral dog study
BPD Data set IIA/ Annex Point VI. 6.4	

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*	-	-
□-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,* = $p < 0.05$; ** = $p < 0.01$

3.12.1.5 Study 5 – 18-Week inhalation rat study

Doc IIIA/Section A 6.4.3	**Repeated dose toxicity**
BPD Data set IIA/ Annex Point VI. 6.4	18-Week inhalation rat study

		Official use only
	Reference	
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	OECD Guideline 413 Subchronic Inhalation Toxicity (1981)	
	US EPA FIFRA § 82-4 Subchronic Inhalation Toxicity (1984)	
GLP	Yes	
Deviations	No	
	MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	250987	
Specification	As given in section 2	
Description	Grey brown/solid crystalline mass (room temperature)	
Purity	95.0%	
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.	
Test Animals		
Species	Rat	
Strain	Bor:WISW (SPF-Cpb) (Wistar)	
Source	Winkelmann, Borchen	
Sex	Male and female	
Age/weight at study initiation	2-3 months Mean body weight at start of study: 190 g (males) and 175 g (females)	
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")	
Control animals	Yes, air control and vehicle control animals	
Administration/ Exposure	Inhalation	
Duration of treatment	13 weeks	
Frequency of exposure	5 days per week	
Postexposure period	4 weeks for satellite groups	
Inhalation		
Concentrations	Nominal: 0, 40, 250, 1000 [mg/m³] Analytical: 0, 4.9, 46.7, 220.2 [mg/m³]	
Particle size	MMAD 1.1 [µm] ± GSD 1.4 [µm]	
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.
Type of exposure	Nose/head only
Vehicle	Polyethylene glycol E 400/ethanol (1:1)
Concentration in vehicle	0.4, .5, and 10 % (w/v)
Duration of exposure	6 h
Controls	Sham exposed and vehicle exposed
Examinations	
Observations	
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 4^{th}, 8^{th} and 12^{th} week the following reflexes were tested: cornea, pinna, myotactic, light, startle, and righting
Mortality	Yes, observed twice daily.
Body weight	Yes, before study, then weekly.
Food consumption	Yes, calculated weekly.
Water consumption	No
Ophthalmoscopic examination	Yes, before start of study and week 17, 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.
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*.
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, $\alpha 1$, $\alpha 2$, β and γ globulins), fluoride (in bones and teeth).
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.
Sacrifice and pathology	
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.
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.
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
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."
Further remarks	
	Results and Discussion
Observations	
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.
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.
Body weight gain	No treatment related effects were seen.
Food consumption and compound intake	No treatment related effects were seen on food consumption or compound intake.
Ophthalmoscopic examination	No effects
Blood analysis	
Haematology	Females in the high dose group had statistically significant increased percentages of polymorphonuclear neutrophils. No other treatment related effects were seen.
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.
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.
Sacrifice and pathology	
Organ weights	No treatment related effects were seen.
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.
Other	Enzyme induction: No treatment related effects were seen. Pulmonary function: No treatment related effects were seen.
	Applicant's Summary and conclusion
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).

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 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.
Conclusion	
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.
NO(A)EL	The no observable adverse effect level is 46.7 mg/m^3, approximately equivalent to 16.8 mg/kg bw/day.
Other	
Reliability	1
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/m^3		46.7 mg/m^3		220.2 mg/m^3 [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.
* = $p < 0.05$; ** = $p < 0.01$

3.12.1.6 Study 6 – 2-year oral rat study

Doc IIIA/Section A 6.5/01 **Chronic Toxicity / Carcinogenicity**
BPD Data set IIA/ Annex Point VI. 6.5/6.7 2-year oral rat study

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		

Doc IIIA/Section A 6.5/01	**Chronic Toxicity / Carcinogenicity**
BPD Data set IIA/ Annex Point VI. 6.5/6.7	2-year oral rat study
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
	Guidelines and Quality Assurance
Guideline study	Yes
	OECD 453 (1981)
	US EPA FIFRA § 83-5 (1984)
GLP	Yes
Deviations	No
	MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
Lot/Batch number	Mixed batch no: 130187, from 10.11.87: 250987
Specification	As given in sections 2 and 3
Description	Brown-yellow clear liquid after heating to 50ºC
Purity	95.0% (130187), 94.5% (250987)
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.
Test Animals	
Species	Rat
Strain	Wistar; Bor:WISW (SPF-Cpb)
Source	Winkelmann, Borchen
Sex	Male and female
Age/weight at study initiation	4-6 weeks
	Weight range at start of study 54-78 g (males) and 52-73 g (females)
Number of animals per group	70 rats/sex/group
At interim sacrifice	10 animals/group/sex at 12 months
At terminal sacrifice	60 animals/group/sex
Control animals	Yes
Administration/ Exposure	Oral (dietary)
Duration of treatment	25 months
Interim sacrifice(s)	After 12 months
Final sacrifice	After 25 months
Frequency of exposure	Daily (continuous in diet)
Postexposure period	None
	Oral
Type	In food
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
Vehicle	Moistened with peanut oil/ mixed into food (1% final concentration)
Concentration in vehicle	N/A
Total volume applied	Not applicable
Controls	Diet with peanut oil
Examinations	
Body weight	Yes, before administration of first dose and then weekly.
Food consumption	Yes, measured weekly.
Water consumption	Yes, measured weekly.

Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
Macroscopic investigations	Location and progression of palpable masses, skin tumours were recorded
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.
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
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
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*
Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at interim and final sacrifice
Organ Weights	Yes, from all animals at interim or final sacrifice Organs: liver, kidneys, adrenals, testes, spleen, brain, heart, and lungs
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.
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.
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.
Further remarks	
	Results and Discussion
Mortality	No treatment-related mortality was observed among all treated groups. Survival rate was equivalent across all groups.
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 weight intermittently throughout the study.
Food consumption	No treatment related effects were seen.
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.
Clinical signs	No treatment related effects were seen
Macroscopic investigations	No treatment related effects were seen.
Ophthalmoscopic examination	No treatment related effects were seen
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.
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.
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.	
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.	
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
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.	
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.	
Time to tumours	No treatment related effects were seen.	
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. Applicant's Summary and conclusion
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).
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 1.0 and 1.4 mg/kg bw-day for males and females respectively).	
Conclusion		X
Reliability	1	
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

	Control data study		**low dose**		**medium dose**		**high dose**		**dose-response + /**	
Parameter	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

	Control data study		**low dose**		**medium dose**		**high dose**		**dose-response + /**	
Parameter	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.

3.12.1.7 Study 7 - 2-year oral mouse study

Doc IIIA/Section A 6.5/02 **Chronic Toxicity/Carcinogenicity**
BPD Data set IIA/ Annex Point VI. 6.5/6.7 2-year oral mouse study

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD 451 (1981) US EPA FIFRA § 82-2 (1984)	

GLP	Yes
Deviations	No
	MATERIALS AND MethodS
Test material	NAK 4455 (transfluthrin)
Lot/Batch number	Mixed batch no: 250987
Specification	As given in sections 2 and 3
Description	Dark brown
Purity	94.5- 95%
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.
Test Animals	
Species	Mice
Strain	B6C3F1
Source	Charles River Wiga GmbH
Sex	Male and female
Age/weight at study initiation	5-6 weeks Weight range at start of study 18-24 g (males) and 15-20 g (females)
Number of animals per group	60 rats/sex/group (+ extra 10 rats/sex/group for 0 and 1000 ppm groups)
at interim sacrifice	10 animals/sex/group at 12 months
at terminal sacrifice	50 animals/sex/group
Control animals	Yes
Administration/ Exposure	Oral
Duration of treatment	24 months
Interim sacrifice(s)	After 12 months
Final sacrifice	After 24 months
Frequency of exposure	Daily
Postexposure period	None
	Oral
Type	In food
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
Vehicle	Moistened with peanut oil/ mixed into food
Concentration in vehicle	Not applicable
Total volume applied	Not applicable
Controls	Diet with peanut oil
Examinations	
Body weight	Yes, before administration of first dose and then weekly.
Food consumption	Yes, measured weekly (based on extra 10 animals).

Water consumption	Yes, measured weekly.
Clinical signs	Yes, observed twice daily, in particular body surfaces, body orifices, posture, general behaviour, respiration and excretory products.
Macroscopic investigations	Palpable masses, skin tumours
Ophthalmoscopic examination	No.
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)
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
Urinalysis	No
Pathology	Yes, all animals which died spontaneously or were moribund and sacrificed, all animals at 3-month, interim and final sacrifice
Organ Weights	Yes, from all animals at 3-month, interim or final sacrifice Organs: liver, kidneys, testes, spleen, brain, heart, ovaries, and lungs
Histopathology	Yes, 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 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.
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.
Further remarks	
	Results and Discussion
Mortality	No treatment-related mortality was observed among all groups.
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.
Food consumption	No treatment related effects were seen.
Water consumption	No treatment related effects were seen.
Clinical signs	No treatment related effects were seen,
Macroscopic investigations	No treatment related effects were seen.
Ophthalmoscopic examination	Not applicable
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).
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.
Urinalysis	Not applicable.
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.
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.
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.
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.
Time to tumours	Not applicable
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.
	Applicant's Summary and conclusion
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.
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.
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.*
Reliability	1
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
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**	
Chol ester ol, 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)	-	-	-	-	↑*	↑	↑**	↑**	+	+
Relative weight (interim 12 month)	-	-	-	-	-	↓**	↑**	-	-	-
Relative weight (final 24 month)	-	-	-	-	-	-	↑**	↑**	-	-

*p <0.05, ** p<0.01, *** P<0.001, - 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**	-	-

3.12.1.8 Study 8 - 1 year oral dog study

Doc IIIA/Section A 6.5/03	**Chronic toxicity**
BPD Data set IIA/ Annex Point VI. 6.5	1 year oral dog study

	Reference	**Official use only**
Data protection	Yes	
Data owner	BAYER AG	
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD Guidelines 452 (1981)	
GLP	Yes	
Deviations	None which affected the integrity of the study. MATERIALS AND MethodS	
Test material	NAK 4455 (transfluthrin)	
Lot/Batch number	250987	
Specification	As given in sections 2 and 3	
Description	Solid	
Purity	95.1%	
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.	
Test Animals		
Species	Dog	
Strain	Beagle	
Source	F. Winkelmann	
Sex	Male and female	
Age/weight at study initiation	21-25 weeks old 5.9-9.7 kg	
Number of animals per group	4 dogs/sex/group	
Control animals	Yes	
Administration/ Exposure	Oral (dietary)	
Duration of treatment	1 year (364 or 365 days)	
Frequency of exposure	Daily	
Postexposure period	None	
Oral		
Type	In food	
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)	
Vehicle	Test compound mixed with pulverised dry feed and moistened with water	
Concentration in vehicle	Not applicable	
Total volume applied	Not applicable	

Controls	Pulverised dry feed moistened with water
Examinations	
Observations	Animals were inspected several times daily.
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
Mortality	Yes, observed daily
Body weight	Yes, weekly.
Food consumption	Yes, recorded daily.
Water consumption	Not recorded
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.
Haematology	Yes, in weeks -2, 3, 7, 13, 20, 26, 39 and 52. Blood samples were 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.
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.
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
Sacrifice and pathology	All animals were sacrificed by exsanguination at the end of the study under Evipan anaesthesia, dissected and grossly examined.
Organ Weights	The following organs were weighed: liver, kidneys, adrenals, testes, spleen, brain, heart, ovaries, pancreas, prostate gland, thyroid gland, pituitary and lung.
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 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.
Other examinations	None
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.
Further remarks	
	Results and Discussion
Observations	
Clinical signs	No treatment related effects on general health, reflexes, temperature or pulse were observed.
Mortality	No mortalities were seen.
Body weight	There was no treatment related effect on body weight.
Food consumption and compound intake	No treatment related effects were seen on food consumption or compound intake.
Ophthalmoscopic examination	No treatment related effects.
Blood analysis	
Haematology	There were no treatment related effects.
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 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.
Urinalysis	No treatment related effects were seen.
Sacrifice and pathology	
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.
Gross and histopathology	No treatment related findings were noted.
Other	None
	Applicant's Summary and conclusion
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.
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.
Conclusion	

Doc IIIA/Section A 6.5/03	**Chronic toxicity**
BPD Data set IIA/ Annex Point VI. 6.5	1 year oral dog study
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.
NO(A)EL	The no observable adverse effect level in this study is 300 ppm (10 mg/kg bw/day).
Other	
Reliability	1
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	*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

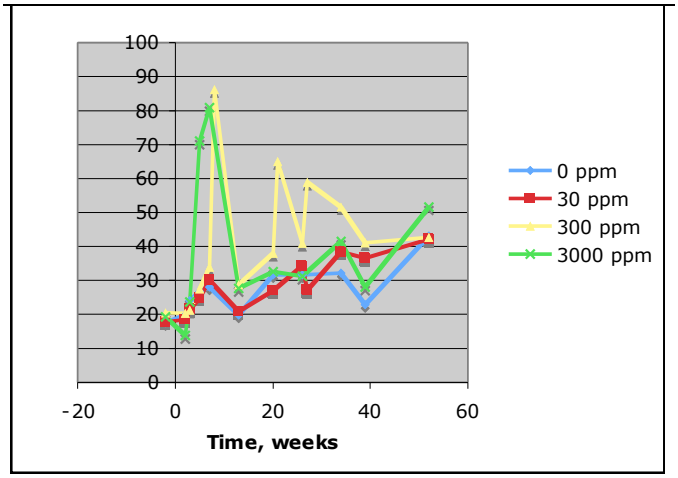


Figure A6_5.3-2 APh levels in dogs after chronic NAK 4455 administration

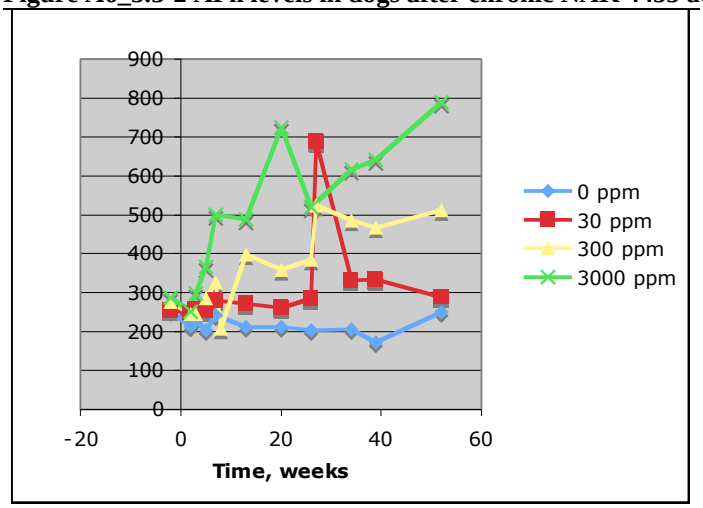


Figure A6_5.3-3 GLDH levels in dogs after chronic NAK 4455 administration

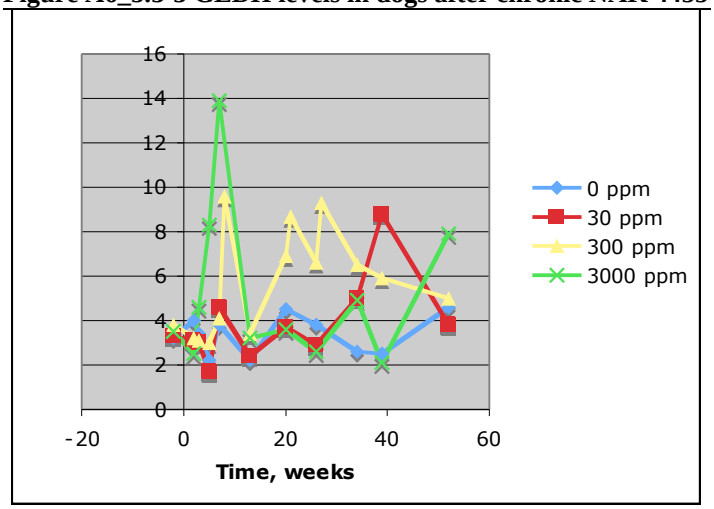


Figure A6_5.3-4 Cholesterol levels in dogs after chronic NAK 4455 administration

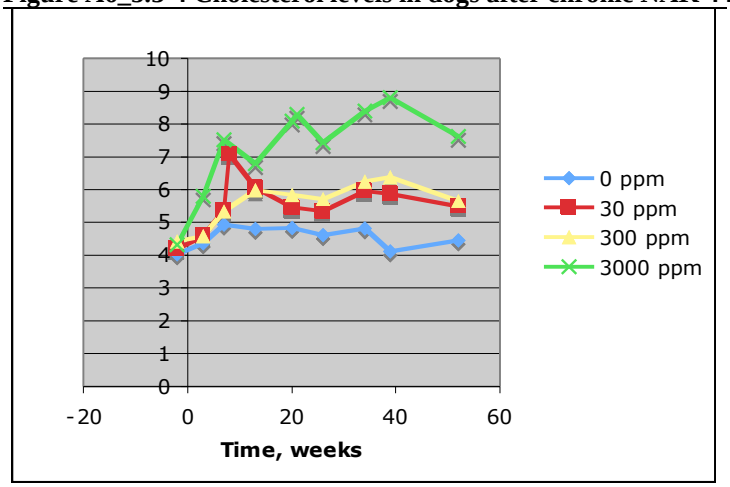


Figure A6_5.3-5 Serum triglyceride levels in dogs after chronic NAK 4455 administration

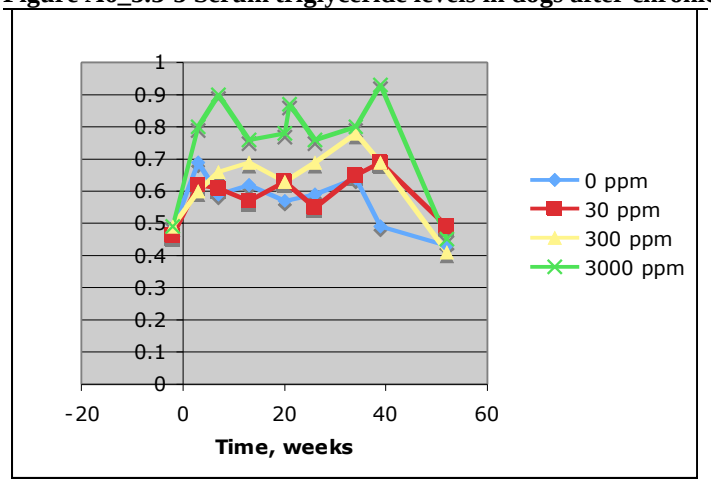


Figure A6_5.3-6 Bilirubin levels in dogs after chronic NAK 4455 administration

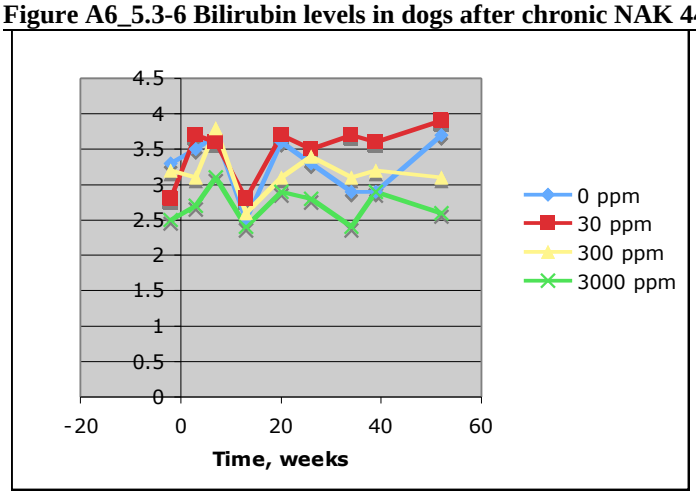


Figure A6_5.3-7 Hepatic N-demethylase levels in dogs after chronic NAK 4455 administration for 52 weeks

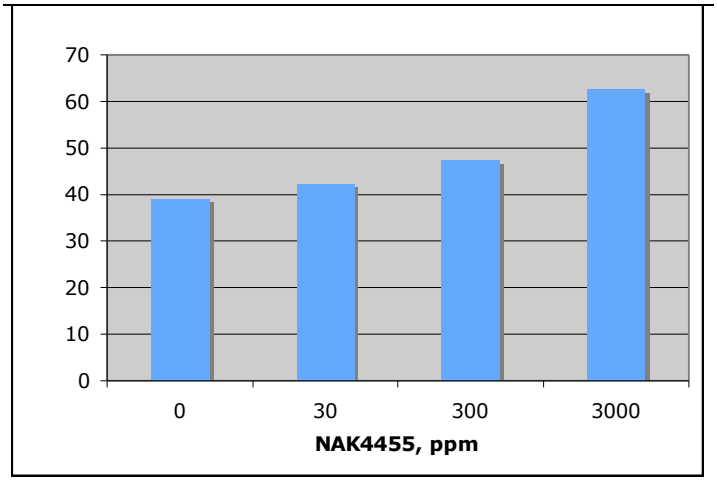
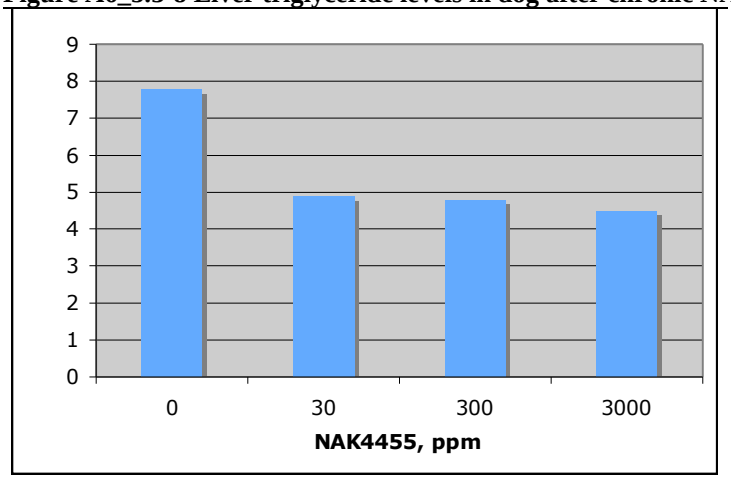


Figure A6_5.3-8 Liver triglyceride levels in dog after chronic NAK 4455 administration for 52 weeks



3.12.2 Human data

No data available.

3.12.3 Other data

No data available.

3.13 Aspiration hazard

3.13.1 Animal data

No data available.

3.13.2 Human data

No data available.

3.13.3 Other data

No data available.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Document IIIA/ Section A7.1.1.2.1 **BPD Data set IIA/ Annex Point VII.7.6.1.1**	**Biodegradability (ready)**	
	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes OECD 301 F Manometric Respirmetry	
GLP	No. GLP was not compulsory at the time the study was performed	
Deviations	The report lacks experimental detail to determine the methodology used in the test. The study claims compliance with OECD 301F, so it can be assumed that the correct methodology was used. Several deficiencies are apparent including: Purity/batch of test material not stated Details on mineral medium used in test lacking. No abiotic control included. No indication if the incubations were replicated or not. pH not	

	measured. Although the study lacks experimental and reporting detail, it has been conducted according to a recognised guideline and can be considered a valid for risk assessment purposes. Materials and Methods	
Test material	Transfluthrin	
Lot/Batch number	Not stated	
Specification	Presumably as given in section 2 of Doc IIIA.	
Purity	Not stated	
Further relevant properties	Low water solubility: 0.057 mg/l	
Composition of Product	Not applicable.	
TS inhibitory to microorganisms	No information	X
Specific chemical analysis	None	
Reference substance	Yes, Aniline	
Initial concentration of reference substance	100 mg /l	
Testing procedure		
Inoculum / test species	See table A7.1.1.2-2	
Test system	See table A7.1.1.2-3	
Test conditions	See table A7.1.1.2-4	
Method of preparation of test solution	Test substance used as supplied and added by direct weighing.	
Initial TS concentration	100 mg test substance/L.	
Duration of test	28 days	
Analytical parameter	Oxygen consumption (this was related to the Theoretical Oxygen Demand (TOD) and concentration of the test substance and expressed as per cent biodegradation rate.	
Sampling	4, 6, 8, 12, 14, 20, 22, 26 and 28 days.	
Intermediates/ degradation products	Not identified.	
Nitrate/nitrite measurement	No	
Controls	Control without test substance and procedure control (aniline reference).	
Statistics	Not stated	

Results

Degradation of test substance

Graph: Zero degradation was observed after 28 days, therefore no graphical plot is considered necessary.

Degradation: Zero degradation (calculated to the theoretical oxygen demand of 1336 mg O_2/g) was observed after 28 days.

See table A7.1.1.2-5

Other observations: None

Degradation of TS in abiotic control: Not determined.

Degradation of reference substance: Percentage degradations of Aniline are presented below;

Day No.	% Biodegradation of Aniline
4	0
6	20
8	70
12	77
14	78
20	79
22	80
26	80
28	80

Intermediates/ degradation products: Not identified.

Applicant's Summary and conclusion

Materials and methods: Test substance: Transfluthrin (batch and purity not given). The test was conducted according to OECD Guideline 301F Manometric Respirometry Test.

Transfluthrin was suspended in a mineral medium, inoculated with a mixed population of aquatic organisms (activated sludge conc in flasks: 30 mg ss/l) at a rate of 100 mg/L. The reference material (aniline) was added to the same rate of 100 mg/L.

	Test bottles for control, procedure control (aniline reference) and test substance were incubated in the dark at 20 ± 1ºC for periods of up to 28 days. Oxygen concentration was measured at days 4, 6, 8, 12, 14, 20, 22, 26 and 28 using a respirometer. This was then related to Theoretical Oxygen Demand (1336 mg O_2/g) and concentration of the test substance or reference material and expressed as per cent biodegradation rate.	
Results and discussion	*The degradation rate calculated for oxygen consumption was zero percent in the test suspension after 28 days under the test conditions. From these results it is considered that transfluthrin was not readily biodegradable under the conditions of the test. Degradation of reference material (aniline) calculated from oxygen consumption was 78% within 14 days exposure.*	
Conclusion	*Transfluthin was not readily biodegradable under the conditions of the test. The test met all validity criteria.*	X
Reliability	*2*	
Deficiencies	*Yes*	
	Report lacks experimental detail to determine methodology used. Study claims compliance with OECD 301F, so it can be assumed that the correct methodology was used. Several deficiencies are apparent including:	
	Purity/batch of test material not stated	
	Mineral medium details lacking, toxicity control not included, whether the incubations were replicated or not.	
	Although the study lacks experimental and reporting detail, it has been conducted according to a recognised guideline and can be considered a valid for risk assessment purposes.	

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*09-02-07*

Materials and Methods	*Applicant's version adequately reflects the study report.with the following additional information submitted by the applicant:* *The oxygen consumption in the blank vessel is higher than in the vessel containing the test substance, which potentially indicates a microbial inhibitory effect of transfluthrin. This is contradicted by the results shown in an activated sludge respiration test (Doc IIIA7.4.1.4), which demonstrates that transfluthrin was not inhibitory at concentrations up to 10 000 mg/L. At present there is no scientific explanation for the observed effect at 100 mg/L in the underlying study. There was no assessment of the toxicity of the test substance towards the bacteria (no toxicity test, containing both the test substance and a reference compound, was included in the experiment). In addition no measure of pH is available at the start or the end of the experiment. It can be noted that lower oxygen consumption is observed in only one of the 2 bottles for the test substance. In this bottle the oxygen consumtion reached a maximum at day 12 and then remained stable until the end of the experiment. However the conclusion that transfluthrin is not readily biodegradable remains.* Two bottles were used for each test substance, the reference substance and the control. Only one measure was performed for each bottle.
Results and discussion	*Applicant's version adequately reflects the report, but the following remark should be made:* *The study was performed at 100 mg/L, which is > 1750 x the reported water solubility (57 µg/L). It is possible that strong sorption to particles and/or vessels has influenced the results.. The test was performed according to the guidelines, and the test concentration itself is thus not a reason to consider the test as not reliable. However, it is not possible to draw a definitive conclusion with as to whether the classification* not readily biodegradable *is correct or is due to the test conditions.*
Conclusion	*It is recognised that the study does not meet the current standards with respect to reporting materials and methods and results. However, since the result* not readily biodegradable *reflects a* worst case *classification regarding the risk assessment, repetition of the experiment is not considered necessary.*
Reliability	*2* *The following deficiencies were noted in addition to those reported by the applicant:* *The study was performed at 100 mg/L, which is far above the reported water solubility (57 µg/L).*
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 A7.1.1.2-1: Guideline-methods of EC and OECD for tests on ready/inherent biodegradability (according to OECD criteria); simulation test

Test	EC-method	OECD-Guideline	Test on ready/inherent biodegradability
Manometric Respirometry	C.4-D	301F	ready

Table A7.1.1.2-2: Inoculum / Test organism

Criteria	Details
Nature	*Activated sludge.*
Species	*Not stated.*
Strain	*Not stated*
Source and sampling site	*Laboratory scale unit receiving sewage from the south Wupper area water authority (predominantly domestic sewage)*
Laboratory culture	*No*
Method of cultivation	*N/a*
Preparation of inoculum for exposure	*Not stated.*
Pretreatment	*No*
Initial cell concentration	*Concentration of activated sludge in test flasks: 33 mg ss/L.*

Table A7.1.1.2-3: Test system

Criteria	Details
Culturing apparatus	*Test bottles*
Number of culture flasks/concentration	*Number of replicates not stated.*

	At least one flask for the test suspension and one each for the procedure control and blank control.
Aeration device	*None*
Measuring equipment	*Oxygen consumption was measured using a respirometer.*
Test performed in closed vessels due to significant volatility of TS	*No*

Table A7.1.1.2-4: Test conditions

Criteria	Details
Composition of medium	*Not stated, but assumed to be of a composition stated within OECD 301F.*
Additional substrate	*None.*
Test temperature	*20 ± 1ºC*
pH	*Not stated*
Aeration of dilution water	*Not stated.*
Suspended solids concentration	*Not stated.*
Other relevant criteria	*None*

Table A7.1.1.2-5: Results for degradation

Day	Oxygen consumption (mg O_2/g)		Biodegradation (%)
	Transfluthrin	Blank	
4	19	19	0
6	23	25	0
8	26	28	0
12	30	35	0
14	32	36	0
20	36	43	0
22	37	45	0
26	38	47	0
28	39	49	0

Table A7.1.1.2-6: Pass levels and validity criteria for tests on ready biodegradability

	fulfilled	not fulfilled
Pass levels		
70% removal of DOC resp. 60% removal of ThOD or $ThCO_2$		No
Pass values reached within 10-d window (within 28-d test period) - not applicable to MITI-I-Test - 14-d window acceptable for Closed-Bottle-Test		No
Criteria for validity		
Difference of extremes of replicate values of TS removal at plateau (at the end of test or end of 10-d window) < 20%		No (no replication)
Percentage of removal of reference substance reaches pass level by day 14	Yes	

4.1.2 BOD_5/COD

No data available.

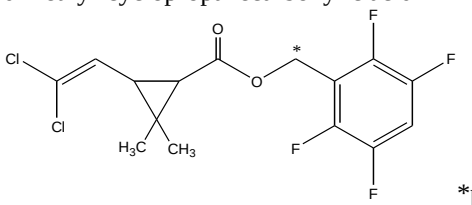
4.1.3 Aquatic simulation tests

No data available.

4.1.4 Other degradability studies

4.1.4.1 Study - Hydrolysis

Document IIIA/ Section A7.1.1.1.1 **BPD Data set IIA/ Annex Point VII7.6.2.1**	**Hydrolysis as a function of pH and identification of breakdown products**	
	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes EPA Pesticide Assessment Guidelines, Subdivision N: § 161-1 (1982)	
GLP	Yes	
Deviations	The method is generally in compliance with the prescribed EU method C.7. Differences included: Rather than using individual hydrolysis vessels, due to the low solubility of the test material and problems encountered in the preliminary experiment with adsorption to the small test vessel walls, a single large reaction vessel was used at each pH. Details of the preliminary study were not reported. The preliminary test only serves as a screen for molecules that are rapidly hydrolysed, therefore the lack of reported detail is not considered to be a significant deficiency. No replication of the hydrolysis experiment was conducted. No deviations are considered sufficient to affect the overall scientific validity of the results obtained. Materials and Methods	
Test material	Transfluthrin (cited as benfluthrin in this report)	

	2,3,5,6-tetrafluorphenyl)methyl ester 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropanecarboxylic acid	
Lot/Batch number	 *position of radiolabel Specific activity: 3.90 MBq/mg Tetrafluorophenyl-[^{14}C-methylene]-transfluthrin: / Batch no.: not given	X
Specification	See purity (Section 3.1.3).	
Purity	Radiochemical purity of the test substance was confirmed by radio-HPLC (98.7%) and radio-TLC (>99%) The chemical purity and identity of the chemical substance were verified by GC-MS.	
Further relevant properties	Water solubility is low: 57 µg/L, therefore a co-solvent, acetonitrile, was used at 1% v/v.	
Reference substance	Transfluthrin (NAK 4455); batch 880325ELB01 (Bayer AG, Elberfeld). Purity: 97.8% (GC), cis-transfluthrin (NAK 4711), trans-transfluthrin (NAK 5368), 2,3,5,6-tetrafluorobenzyl alcohol (NAK 4452), trans-permethrinic acid.	
Test solution	The preparation detail and experimental conditions are summarised in Tables A7.1.1.1.1-1 to A7.1.1.1.1-3. Batches (250 ml) of 0.01M pH 5 acetate buffer, 0.02M pH 7 phosphate buffer and 0.01M pH 9 borate buffer were prepared and steam sterilised. The test material was prepared in acetonitrile at a concentration of 0.391 mg/ml (= 1.524 MBq/ml). Note: The report also cites the buffer concentration as 0.01M, but the description of the buffer preparation in Appendix 3 cites 0.02M, so this value has used through out the summary.	
Testing procedure		
Test system	The test system is detailed in Table A7.1.1.1.1-3. Based on the results of a preliminary experiment, in which pronounced adsorption of the active substance to the walls of the test vessels was observed, single batches of pH 5, 7 and 9 buffer (200 ml) were prepared and treated with ^{14}C-transfluthrin in large vessels. To minimise the risk of microbial contamination, subsamples were taken at only 6 time points. 200 ml of each of the steam sterilised buffers were transferred into three 250 Erlenmeyer flasks. 2ml of each buffer was removed and replaced with acetonitrile to give a co-solvent concentration of 1% v/v. The solutions were sonicated (15 mins) and left overnight at room temperature. ^{14}C-transfluthrin was then added to each test vessel at a rate of 19.6 µg (76 kBq) in 50 µl of acetonitrile (i.e. final solution concentration of 98 µg/l). The solutions were wrapped in aluminium foil, sonicated before incubating at 25ºC in the dark for three hours (the time required for uniform distribution of the test material and considered to be time zero, T=0). Subsamples of the buffered test solution were taken for analysis (as described in 3.4.6) at 0, 7, 15, 22/23, 29/30 and 36 (at pH 7 and 9	X

	only) days. Note: due to microbial contamination and loss of sterility in the pH 7 and 9 test solutions, these incubations were repeated.	
Temperature	25 ± 0.1°C	
pH	pH 5: 5.09 (Day 15), 5.11 (Day 30) pH7: 7.09 (Day 15), 7.09 (Day 29), 7.06 (Day 36) pH9: 9.03 (Day 15), 8.96 (Day 29), 8.98 (Day 36)	
Duration of the test	Up to 36 days	
Number of replicates	One	
Sampling	Details of sampling times give in Table A7.1.1.1.1-4. At each sample point, triplicate 5 ml aliquots were removed from the buffered treated solutions under sterile conditions. One aliquot was frozen, one taken for pH measurement and the other for "mass balance" and analysis. From the "mass balance" subsample, a 1ml subsample was taken for quantification by LSC. The remaining solution (4ml) was extracted with 5ml of dichloromethane.	
Analytical methods	Quantification of the dichloromethane extracts was by normal phase TLC using two solvent systems (toluene/methanol, 9/1; chloroform/diisopropylether/acetone, 5/4/1) with evaluation by radio thin-layer analyser. Identification of degradation products was by co-chromatograph with authentic reference standards. GC-MS was also used to identify products isolated by preparative TLC. GC-MS system: HP 5970 (MSD) with GC 5880 A; column: 25m ULTRA, i.d. 0.2 mm, film thickness: 0.25 µm; column head pressure: 10 psi He, injector temp: 200ºC; splitless injection; temp program: 80ºC isothermal (1 min) then to 280ºC at 10ºC/min.	
Preliminary test	Yes Buffer solutions summarised in Table A7.1.1.1.1-1. The preliminary test demonstrated that the solubility of NAK 4455 was below 100 µg/l even when the maximum concentration of co-solvent is used. Pronounced adsorption of the test material to the walls of the test vessels was observed in the preliminary experiment, which dictated the design of the main incubation.	
	Results	
Mass balance results	At pH 5, relative to the total recovered radioactivity in the T=0 sample, 98.8 - 102.1% of the radioactivity was recovered.	X
	At pH 7, relative to the total content of the T=0 sample, at least 93.5% of the radioactivity were recovered in samples taken up to day 29. Recovery of radioactivity in the terminal day 36 sample was only 80.2%. It was assumed that this loss was due to incomplete recovery of adsorbed radioactivity at this particular time point.	X X
	At pH 9, relative to the T=0 sample, 98.7 – 103.2% AR relative to the T=0 samples was recovered. Sterility of the samples was maintained at pH 5, but was compromised on Day 15 in the pH 7 and 9 solutions. Theses incubations were repeated. The sterility was maintained in these latter incubations.	

Hydrolysis rate constant

The concentration of transfluthrin determined at T=0 was equated to 100%, the degradation rates were calculated relative to this point.

Sample set	Degradation rate constant ($days^{-1}$)	Half-life (days)	r^2
pH 5	0.0001	> 1 year	-
pH 7	0.0007	> 1 year	-
pH 9	0.049	14	0.9818

Dissipation time

DT_{50} values are summarised in 4.2

Concentration – time data

See table A7.1.1.1.1-4.

Specification of the transformation products

See table A7.1.1.1.1-4. At pH 5 no degradation products were present at > 1% AR (applied radioactivity) during the 30 day hydrolysis period.
At pH 7 and 9 there was formation of one degradate (maxima: 3.5% AR pH 7; 81.9% AR pH 9). Analysis by co-chromatograph and GC-MS of the isolated degradate identified the only major product as 2,3,5,6-tetrafluorobenzylalcohol (denoted as NAK 4452 in the original report).

Applicant's Summary and conclusion

Materials and methods

Based on the results of a preliminary experiment, single batches of sterile pH 5, 7 and 9 buffer (with 1% co-solvent (acetonitrile)) were treated with tetrafluorophenyl-[^{14}C-methylene]-transfluthrin at a nominal rate of 98µg/l and incubated at 25 ± 0.1ºC for periods of up to 36 days. At days 0, 7, 15, 22/23, 29/30 and 36 (pH 7 and 9 only), triplicate 5 ml subsamples were removed from each treated buffer solution. One replicate was analysed for pH, one frozen and the other quantified by direct LSC and checked for sterility. After quantification by LSC, the remaining sample was extracted with dichloromethane and the organic phase analysed by TLC. The major degradate was isolated at pH 9 and identified by co-chromatography and GC-MS.

Results and discussion

The radiochemical purities of the test substances were determined to be >98% by TLC prior to experimental start and time zero samples showed that the test material was stable under the conditions of administration.
The pH of the samples was measured at each sampling time. The results for all sets of samples indicated that the buffering capacity was maintained in the solution during the study period.
Sterility assays at pH confirmed sterility over the 30 – 36 day incubation period. At pH 7 and 9, the sterility was compromised at day 15, so these incubations were repeated. Subsequent sterility checks confirmed sterility at pH 7 and 9 over 36 days.
Total material balance recoveries ranged from 80.2 to 103.2% AR. Degradation of tetrafluorophenyl-[^{14}C-methylene]-transfluthrin was minimal at pH 5 and 7 at 25 ±0.1°C. At pH 5, benfluthirn (transfluthrin) degraded from an initial 95.79% AR to 94.12% AR. At pH 7, transfluthrin degraded from 97.41% AR to 75.21% after 35 days. The latter value was due to poor recovery of radioactivity rather than

degradation, as only one degradate was present, accounting for a maximum of 3.5% AR.
At pH 9, transfluthrin degraded from an initial 97.92% AR to 17.8% AR over 35 days. One major degradate was formed, which accounted for a maximum of 81.88% AR after 35 days. This was identified by co-chromatography and GC-MS as 2,3,5,6-tetrafluorobenzylalcohol.

k_H, DT_{50}, r^2

Sample set	Degradation rate constant ($days^{-1}$)	Half-life (days)	R^2
pH 5	0.0001	> 1 year	-
pH 7	0.0007	> 1 year	-
pH 9	0.049	14	0.9818

Conclusion: Hydrolysis studies showed that transfluthrin was stable to hydrolysis at pH 5 and pH 7 at 25°C with half-lives estimated to greater than one year. The half-life of transfluthrin at 25°C at pH 9 was determined to be 14 days, using pseudo first order kinetics. The major degradate observed was 2,3,5,6-tetrafluorobenzylalcohol.

Reliability: 2

Deficiencies: Due to technical difficulties, one batch of each test buffer was prepared, rather than individual test vessels and no repetition of the hydrolysis was conducted.
Quantification of the hydrolysis product at pH 7 lacked detail, but as this product was the major product at pH 9 and was subsequently identified by co-chromatography and GC-MS, this lack of detail is not considered to be a significant deficiency.
Considering the hydrolytic stability of transfluthrin at the environmentally relevant pHs of 5 and 7, it is considered that the study is adequate to assess its hydrolytic behaviour.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	08-02-2007
Materials and Methods	Applicant's summary is adopted with the following additions: 3.4.1 time 0 is 3 hours after addition of the test substance 4.1 recovery by adding up radioactivity of all samples taken and of the remaining batch was 100, 88 and 102 % of the theoretically applied amount.
Results and discussion	Applicant's version is adopted.
Conclusion	Applicant's version is adopted. The results no hydrolysis within 30 days at pH 5 and 36 days at pH 7, and DT_{50} 14 days at pH 9 are accepted for risk assessment. Recalculated to 12°C the DT_{50} is 40 days at pH 9, with no hydrolysis at lower pH.
Reliability	2 See applicant's summary for deficiencies.
Acceptability	Acceptable Most of the deficiencies are considered to be inevitable due to the specific characteristics of the test substance.

Remarks	Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity is acceptable.
	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 A7.1.1.1.1-1: Type and composition of buffer solutions

pH	Type of buffer (final molarity)	Composition
5	0.02 M	Sodium acetate: 125 ml of a 0.04 M sodium acetate solution (5.44 g $NaC_2H_3O_2$ x 3 H_2O/l) was brought to a volume of 250 ml with purified water (see Table A7.1.1.1.1-2 for water specification). The pH was adjusted to pH 5 at 25 ± 2°C using 0.04M sodium hydroxide or acetic acid.
7	0.02 M	Potassium dihydrogen phosphate: To 125 ml of a 0.04 M Potassium dihydrogen phosphate solution (5.44 g KH_2PO_4/l) 74 ml of a 0.04M sodium hydroxide solution was added (1.6 g NaOH/l) and diluted to 250 ml with water). The pH of this solution was adjusted to pH 7 at 25 ± 2°C using 0.04M sodium hydroxide solution or phosphoric acid.
9	0.02 M	0.04 mole boric acid (2.48 g H_3BO_4) are dissolved in 1 litre of a 0.04M potassium chloride solution (2.98 g KCl/l). To 125 ml of this solution was added 53 ml of a 0.04 M sodium hydroxide solution (1.6g NaOH/l) and diluted with water to 250ml. the pH of this solution was adjusted to pH 9 at 25 ± 2°C using 0.04M sodium hydroxide solution or boric acid.

Buffered solutions were sterilised using steam sterilisation prior to incubation.

Table A7.1.1.1.1-2: Description of test solution

Criteria	Details
Purity of water	Water was purified through a Milli-Q unit (MILLIPORE) with a bacterial filter. All other

	chemical were reagent quality.
Preparation of test medium	50 µL of dose solution was added to 200 mL of buffer solution. Dosing was undertaken under aseptic conditions in a biological hood flow cabinet.
Test concentrations (mg a.i./L)	Nominal dose rate of 98 µg/L. Measured: 82 – 95 µg/L
Temperature (□C)	25°C ± 0.1 for up to 36 days.
Controls	Not applicable.
Identity and concentration of co-solvent	Acetonitrile at 1% v/v to enable preparation of a homogenous solution, avoiding high adsorption to glass surfaces.
Replicates	Due to technical difficulties, dingle replicate were used.

Table A7.1.1.1.1-3: Description of test system

Glassware	Sterile 250 mL glass Erlenmeyer flasks with glass stoppers.
Other equipment	pH of all buffer solutions measured with an Orion 501 pH meter. Dosing under aseptic conditions in a biological hood flow cabinet. Mixing of dosed solution using a Wrist Action Shaker. Test systems were maintained in a temperature controlled room or water bath in the dark (wrapped with aluminium foil to minimise light exposure). TLC (2 solvent systems) used to analyse sample solutions (parent and metabolites quantification). Preparative TLC was used to isolate degradates of interest which were analysed by GC-MS
Method of sterilization	Steam sterilisation

Table A7.1.1.1.1-4: Hydrolysis products of tetrafluorophenyl-[^{14}C-methylene]-transfluthrin (expressed as % AR)

	Sample time (days)				
	0	**7**	**15**	**22**	**30**
pH 5					
Transfluthrin	95.79	93.56	97.05	94.67	94.12
NAK 4452	*	*	*	*	0.25

	Sample time (days)					
	0	**7**	**15**	**23**	**29**	**36**
pH 7						
Transfluthrin	97.41	92.00	92.96	91.05	89.38	75.21
NAK 4452	*	*	*	*	*	3.56
pH 9						
Transfluthrin	97.92	89.77	49.00	35.57	27.59	17.80
NAK 4452	*	7.69	46.46	60.65	70.86	81.88

NAK 4452 - 2,3,5,6-tetrafluorobenzylalcohol
* Values not reported

Table A7.1.1.1.1-5: Dissipation times of at pH 5, pH 7 and pH 9

	DT50		
	pH5	**pH 7**	**pH 9**
Transfluthrin	estimated > 1 year	estimated > 1 year	14 days

4.1.4.2 Study 2 – Inherent biodegradability

Document IIIA/Section 7.1.1.2.2 BPD Data Set IIA/ Annex Point VII.7.6.1.2	Inherent Biodegradability	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	Transfluthrin was subject to a ready biodegradation test (equivalent to OECD Guideline 301F) and was found not to be readily biodegradable under the conditions of the test (Document III-A, section 7.1.1.2.1). Inherent biodegradability tests allow prolonged exposure of the test compound to microorganisms, or more favourable conditions for biodegradation, however as such, biodegradation under environmental conditions may not be assumed and the tests are not considered to provide adequate information for risk assessment purposes (TGD, Chapter 3, section 7.0.2.2.2). Manufacture and formulation of transfluthrin will be outside the EU therefore it is expected that exposure of the environment will only arise from the use and disposal of transfluthrin products. The proposed uses of the transfluthrin in the EU are for small scale localised use, as domestic (amateur) insecticides both indoors and outdoors e.g. patio use; no direct exposure/contamination of the outdoor environment is anticipated (see Doc IIB, section 3.3). Detailed environmental exposure assessments have been carried out, assuming a worst case scenario of no biodegradation in water/sediment systems (although biodegradability under biotic conditions was observed in the submitted water/sediment study). Exposure of the outdoor environment from the proposed uses of transfluthrin is negligible (see Doc IIB, Section 3.3). The amateur indoor use of transfluthrin (from use of Raid Portable Electric), with subsequent deposition and transference of residues from room surfaces to wastewater, results in negligible concentrations in STP (8.8×10^{-10} – 8.8×10^{-7} mg/l) and surface water (1.9×10^{-11} – 8.2×10^{-8} mg/l). Worst case local contamination of outdoor air (from use of Raid Portable Electric) assuming standard room ventilation rates has been estimated to be 1.31×10^{-10} mg/m^3 (100m from source). Worst case contamination of soil *via* atmospheric deposition from use of one coil (Baygon mosquito coil) is estimated to be 6.8×10^{-11} mg/kg (this is considered to negligible and is analytically non-determinable). The estimated atmospheric half-life of transfluthrin for gas-phase reactions with photochemically produced hydroxyl radicals is 19.4 hours (12 hr day) and with ozone 49 days (Document IIIA, section 7.3.1). Therefore during an emission episode (8 hours) some degradation of	

Document IIIA/Section 7.1.1.2.2 BPD Data Set IIA/ Annex Point VII.7.6.1.2	Inherent Biodegradability	
	transfluthrin maybe expected (with up to 25% reduction in concentration). Due to the fact that transfluthrin is not directly emitted to water or soil and importantly there is extremely limited exposure to terrestrial and aquatic environmental compartments, even assuming zero biodegradation in the case of water, the need to conduct a study on inherent biodegradability is considered to be scientifically unjustified.	
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date Evaluation of applicant's justification Conclusion Remarks	*11-02-2007* *Applicant's justification is accepted. In view of the presence of acceptable data on degradation in water/sediment systems, there is no need to perform a study on inherent biodegradability.* *No additional data needed.*	
	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	
Date Evaluation of applicant's justification Conclusion Remarks		

4.1.4.3 Study 3 – Biodegradation in seawater

Document IIIA/Section 7.1.1.2.3 BPD Data Set IIA/ Annex Point XII.2.1	Biodegradation in seawater	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	Detailed human and environmental exposure assessments have been carried out (Document II-B, sections 3.2 and 3.3), taking into account the frequency and duration of use, the emission rate of the active substance from the product. Since there is no direct emission to water and the product uses are small scale and localized, i.e. uses as domestic insecticides for use indoors and outdoors (patio use), no direct exposure/contamination of the environmental compartments is anticipated so the only possible route of entry to water is via cleaning of residues deposited on indoor surfaces, with washings entering a sewerage system via a sink (e.g. washing of the cloth used for cleaning the surface under the tap). The use of transfluthrin in a vapouriser, with subsequent deposition and transference of residues from room surfaces to wastewater, results in negligible concentrations in concentrations in STP (8.8×10^{-10} – 8.8 x	

Document IIIA/Section 7.1.1.2.3 BPD Data Set IIA/ Annex Point XII.2.1	Biodegradation in seawater	
	10^{-7} mg/l) and surface water (1.9 x 10^{-11} – 8.2 x 10^{-8} mg/l). According to the TGD on risk assessment, the dilution factor for discharges to the coastal zone (100) is greater than that applied to the freshwater environment. The estimated Clocal seawater is 8.2 x 10^{-9} to 8.2 x 10^{-12} mg/l. Due to limited exposure to the marine environmental compartment, the need to conduct studies on biodegradation in seawater is considered to be scientifically unjustified.	
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	EVALUATION BY RAPPORTEUR MEMBER STATE *11-02-2007* *Applicant's justification is accepted.* *No additional data needed.*	
Date Evaluation of applicant's justification Conclusion Remarks	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	

4.1.4.4 Study 4 – Aerobic aquatic degradation

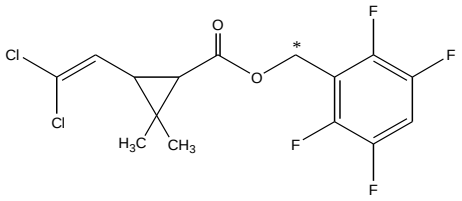
Document IIIA/Section 7.1.2.1.1 BPD Data Set IIIA/ Annex Point XI.2.1	Aerobic biodegradation	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	Manufacture and formulation of transfluthrin will be outside the EU therefore it is expected that exposure of the aquatic environment will only arise from the use and disposal of transfluthrin products. The proposed uses of transfluthrin in the EU are for small scale localised use as domestic (amateur) insecticides both indoors and outdoors e.g. patio use; no direct exposure/contamination of water bodies is anticipated. Detailed human and environmental exposure assessments have been carried out (Document II-B, sections 3.2 and 3.3), 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. Environmental exposure from use and disposal is considered to be negligible. The amateur indoor use of transfluthrin (from use of Raid Portable Electric), with subsequent deposition and transference of residues from room surfaces to wastewater, results in negligible concentrations in STP (8.8 x 10^{-10} – 8.8 x 10^{-7} mg/l) and surface water (1.9 x 10^{-11} – 8.2 x 10^{-8} mg/l). In fact the relatively high log Kow (5.46) and low water solubility (0.0575 mg/L) mean that it is unlikely	X

Document IIIA/Section 7.1.2.1.1 BPD Data Set IIIA/ Annex Point XI.2.1	Aerobic biodegradation	
	that transfluthrin will be present in wastewater (Doc IIB, section 3.3). Indirect photolysis studies (in the presence of humic acid – see Section 7.1.1.1.2) and water-sediment study (see Section 7.1.2.2.2) indicate the rapid removal and abiotic degradation of transfluthrin. Due to limited exposure to aquatic environmental compartments and the biotic degradation demonstrated in the water sediment study (degradation half-life total biotic system in the dark ca. 7-15 days), the need to conduct aerobic biodegradation studies is considered to be scientifically unjustified.	
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*11-02-2007*	
Evaluation of applicant's justification	*Applicant's justification is accepted, except for the statement on photolysis because the photolysis studies are considered not reliable. In view of the presence of acceptable data on degradation in water/sediment systems, there is no need to perform a study on aerobic degradation.*	
Conclusion	*No additional data needed.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

4.1.4.5 Study 5 - Water/sediment degradation study

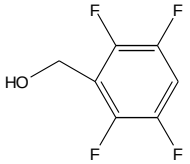
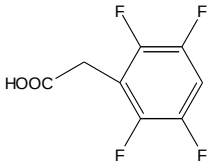
Doc IIIA/Section A7.1.2.2.2 **BPD Data set IIIA/ Annex Point XII.2.1**	**Water/sediment degradation study**

	Reference	**Official use only**
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	

	EPA Pesticide Assessment Guidelines, Subdivision N: § 161-4 (1982)	
	Netherlands Guidelines G 2.1 (1981)	
	Germany, BBA IV, 5-1 (1990)	
GLP	Yes	
Deviations	No	
	The method does not significantly differ from the prescribed method for biocides, OECD 308 (draft)	
	Materials and Methods	
Test material	Transfluthrin (Benfluthrin)	
	2,3,5,6-tetrafluorophenyl)methyl ester 3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane carboxylic acid	
Lot/Batch number		
	*position of radiolabel	
	^{14}C-Transfluthrin:	
	Batch no.: not given	
	Specific activity: 3.90 MBq/mg (105 µCi/mg)	
Specification	As given in section 2 of Doc IIIA	X
Purity	Radiochemical purity of the test substance was confirmed by radio-HPLC (98.7%) and radio-TLC (>99%)	
Further relevant properties	Water solubility:	X
	0.057 ± 2.94 mg/L at 20 °C (Krohn, J - See Doc IIIA Section 3.5)	
	Dissociation Constant	
	Based on its chemical structure, transfluthrin will not dissociate in water (Bogdoll, B and Lemke, G – See Doc IIIA, Section 3.6)	
	Aqueous hydrolysis study	
	The half life of transfluthrin was 14 days at pH 9 and greater than 1 year at pH 5 and 7, assuming pseudo first order kinetics. (Hellpointer, E. – See Document IIIA, Section 7.1.1.1.1),	
	Vapour pressure	

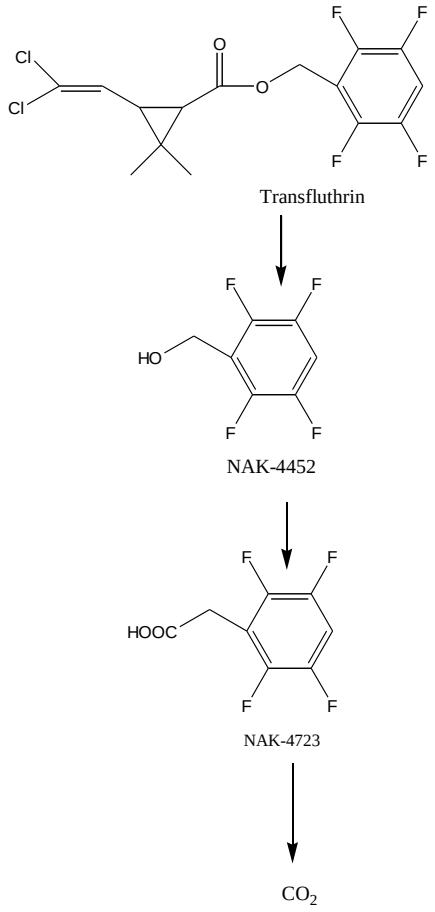
	20°C $9x10^{-4}$ Pa 25°C $2x10^{-3}$ Pa (Weber, R and Krohn, J – See Document IIIA, Section 3.2)	
Reference substances	Transfluthrin (NAK 4455), 2,3,5,6-tetrafluorobenzoic acid (NAK 4723), 2,3,5,6-tetrafluorobenzyl alcohol (NAK 4452)	
Test solution	190 µl of a solution of transfluthrin (41.8 µg) dissolved in ethanol	
Testing procedure		
Test system	Natural sediment (2mm sieved) sourced from Laacherhof (Monheim) and Hönniger Weiher (Wipperfürth), Germnay (physico-chemical details are shown in Table A7.1.2.2.2-1) were placed in glass vessels of 10 cm internal diameter to a depth of 2 cm (ca 200g) and overlain with 6cm (ca 500 ml) depth of 2mm sieved natural water. The surface of the water was stirred/shaken to induce movement, whilst the sediment remained still. Flasks were fitted with appropriate traps containing a polyurethane plug and soda lime to collect volatile compounds and incubated at 20ºC ± 2ºC for periods of up to 100 days.	X X
	The system was equilibrated for 2 weeks under test conditions. The redox potential in the water remained relatively steady over this period (166-174 mV at start to 178 -225mV after pre-incubation; oxygen content stable at 79-85%). The redox potential in the sediment decreased from 157 to 163 mV at the start to 1 to -177mV after pre-incubation, hence the sediment can be considered to anaerobic.	X X
	The redox potentials during the study are shown in table A7.1.2.2.2-2.	
Properties of light source	One set of incubations occurred under light conditions using a mixture of natural sunlight and standard growth lamps. The vessel was fitted with a quartz glass lid (to filter out light of <290 nm). Other incubations were carried out under dark conditions.	
Determination of irradiance	Not stated	
Temperature	20 ± 2 ºC	
pH	The pH values during the study are shown in table A7.1.2.2.2-2.	
Duration of the test	100 days	
Number of replicates	Two replicates per sampling day in dark experiments. One replicate per sampling day in light exposed experiments.	
Sampling	1, 7, 28, 70 and 100 day	
Analytical methods	Water was phase was separated, centrifuged (15 mins, 3300 g),	

	filtered and aliquots taken for LSC. Samples from days 1, 7 and 28 were extracted with dichloromethane. The dichloromethane phase were analysed by TLC. The extracted water phases were freeze dried, the residue reconstituted in methanol and analysed by TLC. Water samples from day 70 and 100 were freeze dried directly after filtration and analysed by LSC (however results suggest that these were also subject to TLC analysis). Sediment was extracted three times with acetonitrile, the extracts filtered, combined and analysed by TLC. The filter residue and extracted sediment residue were air dried prior to combustion and radioassay. Radioactivity in the polyurethane bung was extracted with acetonitrile and then subjected to LSC and TLC analysis. Radioactivity in the soda lime was released with acid, trapped in carbosorb/permafluor and measured by LSC. TLC was undertaken on silica gel plates which were developed with toluene:methanol (9:1) or toluene:hexane (2:1). Results	
Screening test	Not performed	
Radioactive distribution after degradation in test system	Results are shown in Tables A7.1.2.2.2-3, A7.1.2.2.2-4 and A7.1.2.2.2-5 for the systems Laacherhof (dark), Honniger weiher (dark) and Honniger Weiher (light), respectively. In all cases the results were re-presented to include the filter extract that contained transfluthrin adsorbed to the suspended particles, as part of the sediment load.	X
Mean balance of radioactivity	The radioactivity balance was 81.5-106.4% (with one exception where the sample was lost part of the way through processing). Bound residues were 4.4%, 7.9 and 21.8% after 100 days in the Laacherhof, Honniger Weiher (referred to as Hofchen in the kinetic analysis report) and Honniger Weiher (light), systems respectively. Carbon dioxide levels were 3.0, 12.6 and 7.3% after 100 days in the Laacherhof, Honniger Weiher and Honniger Weiher (light), systems respectively. The vast majority of the radioactivity was accounted for by transfluthrin (NAK 4455), NAK 4452, and NAK 4723. The unidentified remaining radioactivity was always <10%.	X
Effects of pH	None observed. However both systems had similar pH.	
4.5 Half-life	Transfluthrin partitioned extremely rapidly into the sediment phase (ca 20% remaining in water after 1 day) and also degraded rapidly in the overall system. First order non-linear regression analysis was used to obtain the DT_{50}/DT_{90} values of transfluthrin in the system from the incubations in darkness. First order non-linear regression analysis was also used to obtain a conservative estimate of the DT_{50}/DT_{90} values of the major metabolite, NAK 4723. This estimation was only based on the decline in amounts over final three time points (28, 77, 100 days) and hence does not consider the	

	effects of ongoing formation of the molecule. All DT_{50}/DT_{90} values obtained are shown in Table A7.1.2.2.2-6.		
Formation and identity of transformation products	Code	% radioactive recovery / Pathway	X
	NAK 4452 (TFB-OH)	Max. 38% in water phase at day 7 / Via ester cleavage	
	NAK 4723 (TBF-COOH)	Max. 59% in water phase and 81.2% in system at 70 day / formed by oxidation of NAK 4452	
Degradation pathway	The proposed degradation pathway is shown in figure 7.1.2.2.2-01.		
	Applicant's Summary and conclusion		
Materials and methods	The behaviour of ^{14}C-transfluthrin in two natural water/sediment systems was investigated under dark and light conditions at 20ºC.		
	Analysis of transfluthrin and transformation products was by one-dimensional thin-layer chromatography. Trapped CO_2 radioactivity was determined by LS measurement.		
Results and discussion	Transfluthrin was degraded rapidly under the conditions of the test and the first order DT_{50} in the whole system was 7-15 days (under dark conditions). The main metabolites were NAK-4455 (maximum 37.8% in the water phase after 7 days) and NAK-4723 (maximum 59.% in the water phase and 81.2% in the whole system after 70 days). The first order DT_{50} for NAK-4723 was conservatively calculated to be 437-485 days.		
	Structure of transformation products:		
	NAK 4452 		
	NAK-4723 		
Conclusion	Transfluthrin was degraded rapidly under the conditions of the test and the first order DT_{50} in the whole system was 7-15 days (under dark conditions). The main metabolites were NAK-4455 (maximum 37.8% in the water phase after 7 days) and NAK-4723 (maximum 59% in the water phase and 81.2% in the whole system after 70 days). The first order DT_{50} for NAK-4723 was conservatively calculated to be 437-485 days.		

Reliability	2
Deficiencies	None.

Figure 7.1.2.2.2-01: Degradation pathway of ^{14}C-transfluthirn in aerobic aquatic sediment systems



Materials and Methods	Applicant's version is adopted with the following additions/amendments: 3.1.2 Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity is acceptable. 3.1.4 0.057 ± **0.00294** mg/L at 20 °C (Krohn, J - See Doc IIIA Section 3.5) 3.3 The amount of ethanol and application rate to test medium are 5.5 ml ethanol in vessel; content approx 700 ml, water phase 200 ml. 3.4.1 The tests were carried out with two different water-sediment systems, which were taken fresh from their natural locations. The water and sediment of Laacherhof origin was taken from a pond in natural state of approx. 500 m^2 without inflow or outflow. The water and sediment of Honniger Weiher origin was taken from an artificially dammed pond in the course of the Honniger Bach river. The pond is approx. 1000 m^2 in size, and has a strong current due to the inflow and outflow. The water passes through the pond into the Dhiinn Valley Dam, which is a drinking water reservoir. Textural class according to USDA is silt loam Sediment content is equivalent to 10 % dwt (0.1 g dwt/mL) for Laacherhof, 14.3 % dwt (0.143 g/mL) for Hönniger Weiher (dark) and 14.6 % dwt (0.146 g/mL) for Hönniger Weiher (light). Applicant gives -177 mV for Hönniger Weiher sediment at end of equilibration, correct value is -117 mV. Redox potential during equilibration specified per system:

	Laacherhof			Hönniger Weiher	
	start	end		start	end
water	166/168	178		167/174	225
sediment	158/159	1		157/163	-117

Applicant states that sediment was anaerobic by the end of the equilibration, but Laacherhof (1 mV) is not. Organic carbon content and texture of both selected sediments is quite similar. Organic carbon content was 5.0% and 3.7%; clay + silt was 70.1% and 75.6% for Laacherhof and Hönniger Weiher, respectively.

3.4.9 Water phase was also analysed for dissolved CO_2, carbonic acid or their salts after addition of HCl.

4.2 Results tables originate from report of Buerkle (2005), and are composed from the data in the original study report. no measured data are available for day 0. In the kinetic evaluation by Buerkle, it is clearly stated that "with only five sampling intervals each suggests that a detailed calculation of the formation/degradation kinetics for each compartment would result in a very high uncertainty of the resulting rate constants and degradation half lives due to the too high number of degrees of freedom"

4.3 Total recovery in individual replicates ranges from **80.9** to 106.4 % of AR; Recovery of radiolabelled material was below the range of 90% to 110% for labelled chemicals.
NAK 4452 = 2,3,5,6-tetrafluorobenzyl alcohol (TFB-OH)
NAK 4723 = 2,3,5,6-tetrafluorobenzoic acid (TFB-COOH)

4.3 Analysis on degradation pathways and transformation products focussed on the benzylmethylene moiety. Information on transformation of the tetrafluorophenyl moiety is limited

4.4 Estimated $DT_{50,system}$ of NAK 4452 (TFB-OH) of < 7 days is based on rapid decline from 2.61 % of AR (Laacherhof) and 39 % of AR (Hönniger Weiher) on day 7 to n.d. on day 28.

4.5 TBF-COOH in text applicant should be changed to TFB-COOH
Maximum formation percentages of metabolites:
NAK 4452 (TFB-OH):
Laacherhof: 4.7 % in water (day 1); 2.5 % in sediment (day 7); 2.5 % in total system (day 7)
Hönniger Weiher: 38 % in water (day 7; large difference between replicates); 2.9 % in sediment (day 7); 39 % in total system (day 7)
NAK 4723 (TFB-COOH):
Laacherhof: 59 % in water (day 70); 24 % in sediment (day 28); 81 % in total system (day 70)
Hönniger Weiher: 55 % in water (day 28); 26 % in sediment (day 28); 82 % in total system (day 28)

Results and discussion	Applicant's version is accepted with the following remarks/additions: The estimated DT_{50} of 437-485 d for metabolite NAK 4723 (TFB-COOH) is not considered reliable, on the basis of the available time points no quantitative estimate is possible. A quantitative estimate for the degradation of this metabolite can not be obtained from the present study. The results for Hönniger Weiher are considered less reliable because of the relatively large difference between the replicates. The $DT_{50,sediment}$ for transfluthrin is estimated by RMS applying non-linear regression of first-order kinetics, using the data as reported by Buerkle (2005): Laacherhof: 17.7 days, r^2 0.9407 Hönniger Weiher: 10.5 days, r^2 0.7592 No additional metabolites are formed in the light exposed systems.
Conclusion	The following results are used for risk assessment: transfluthrin: $DT_{50,water}$ < 7 days, values refers to disappearance due to rapid sorption, $DT_{50,system}$ 14.8 and 7.3 days; $DT_{50,sediment}$ 17.7 and 10.5 days NAK 4452 (TFB-OH): transient in water (max. 38 %), sediment (max. 2.0 %), and total system (max. 39 %). $DT_{50,system}$ < 14 days NAK 4723 (TFB-COOH): maximum formation between day 28 and 70 (59 % in water, 26 % in sediment, 82 % in total system; decline towards end of study (100 days) Bound residues 4.4 and 7.9 % of AR after 100 days for Laacherhof and Hönniger Weiher, respectively. Mineralisation after 100 days was 3.0 and 12.6 % of AR for the respective systems.
Reliability	2 Applies to the combination of both complementary reports. Deficiencies: No information on LOD/LOQ is given. Number of time points (total 5) is small, too small to estimate half-life for major metabolite NAK 4723 (TFB-COOH). For exposure modeling a conservative DT50 of 1000 days can be used Large differences between replicates of Hönniger Weiher.
Acceptability	Acceptable
Remarks	Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity is acceptable.
	Comments from APPLICANT
Date	7 April 2011
Materials and Methods	
Results and discussion	The uncertainties in the pathway with respect to the used methylene label position at the tetrafluorophenyl-part are rather limited since the metabolism of the used radiolabel leads to 3 to 13% of $^{14}CO_2$ at the end of study (100 days). Remaining portions are thus not expected as major metabolites, and are probably either bound residues from a remaining phenol moiety or are released into the air since such compounds might be rather volatile, like it was found for the identified tetrafluorobenzyl alcohol
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A7.1.2.2.2-1 Physicochemical parameters of natural sediments

Sample:	Laacherhof	Honniger Weiher
Textural Analysis (%)		
Sand (63-2000 µm)	29.9	24.4
Silt (2-63 µm)	64.7	72.0
Clay (<2 µm)	5.4	3.6
Class (DIN 19682)	Sandy sludge	Sandy sludge
Organic Carbon (%)	5.0	3.7
pH (in 0.01 M $CaCl_2$)	6.5	5.4
Microbial biomass at study end (mg CO_2/hour/kg sediment)	9	13

Table A7.1.2.2.2-2 pH and Redox potential of the systems during incubation

Day	ID	Laacherhof				Hönniger Weiher			
		pH	Oxygen Content [%]	Redox Potential [mV]		pH	Oxygen Content [%]	Redox Potential [mV]	
				Water	Sediment			Water	Sediment
0	A	8.2	82	178	1	7.8	83	225	-117
	B								
	L								
1	A	8.5	78	119	-14	8.3	74	174	-116
	B	8.5	80	121	-30	8.0	70	205	-99
	L					8.6	95	201	-125
7	A	7.9	56	137	-11	5.5	57	245	-85
	B	7.9	55	199	-31	5.5	55	228	-116
	L					8.1	80	190	-85
28	A	7.9	69	201	70	6.3	66	270	-40
	B	7.9	68	187	59	6.1	60	240	-50
	L					6.2	79	220	-37
70	A	7.3	43	168	-69	5.9	45	180	-85
	B								
	L								
100	A	4.8	85	292	51	7.0	95	230	-29
	B	5.1	79	322	-4	7.4	94	247	-44
	L					6.7	98	255	16

A and B are replicates. L= light exposed

Table A7.1.2.2.2.3 Transfluthrin and its degradates in the water and sediment phases of the system "Laacher Hof" as percentage of applied radioactivity (mean of two replicates)

Water					
Day	transfluthrin in filtered water	Transfluthrin extracted from suspended part (filter)	NAK-4452 in filtered water	NAK-4723 in filtered water	Total CO_2*
1	19.65	5.90	4.74	1.37	0.03
7	0.76	0.42	-	27.45	0.04
28	-	-	-	52.28	0.71
70	-	-	-	59.39	2.05
100	-	-	-	49.88	2.98
Sediment					
	transfluthrin in extracted from sediment	Transfluthrin extracted from sediment and suspended part (filter)	Extracted NAK-4452	Extracted NAK-4723	Non extractable residues*
1	47.05	52.95	-	-	8.08
7	52.36	52.78	2.46	5.68	6.84
28	14.94	14.94	-	23.85	5.31
70	-	7.66	-	21.85	5.45

100	2.27	2.27	-	18.81	4.44

* CO_2 is the sum of that from the sediment and the filtered water. Non-extractable residues is the sum of that from sediment and that in the suspended part (filter)

Table A7.1.2.2.2-4 Transfluthrin and its degradates in the water and sediment phases of the system "Honniger Weiher" as percentage of applied radioactivity (mean of two replicates)

Water					
Day	transfluthrin in filtered water	Transfluthrin extracted from suspended part (filter)	NAK-4452 in filtered water	NAK-4723 in filtered water	Total CO_2*
1	19.72	4.06	6.31	0.85	0.02
7	2.97	0.48	37.67	3.76	0.03
28	-	-	-	55.48	1.52
70	-	-	-	51.27	5.14
100	-	-	-	51.44	12.55
Sediment					
	transfluthrin in extracted from sediment	Transfluthrin extracted from sediment and suspended part (filter)	Extracted NAK-4452	Extracted NAK-4723	Non extractable residues*
1	42.82	46.88	-	-	8.56
7	26.63	27.11	2.94	5.63	4.21
28	10.31	10.31	-	26.12	6.10
70	-	-	-	24.70	7.08
100	2.31	2.31	-	21.16	7.94

* CO_2 is the sum of that from the sediment and the filtered water. Non-extractable residues is the sum of that from sediment and that in the suspended part (filter)

Table A7.1.2.2.2-5 Transfluthrin and its degradates in the water and sediment phases of the system "Honniger Weiher" under light conditions as a percentage of applied radioactivity

Water					
Day	transfluthrin in filtered water	Transfluthrin extracted from suspended part (filter)	NAK-4452 in filtered water	NAK-4723 in filtered water	Total CO_2*
1	12.94	1.27	12.00	2.31	0.03
7	-	-	-	56.73	0.07
28	-	-	-	58.04	0.54
100	-	-	-	42.30	7.27
Sediment					
	transfluthrin in extracted from sediment	Transfluthrin extracted from sediment and suspended part (filter)	Extracted NAK-4452	Extracted NAK-4723	Non extractable residues*
1	46.82	48.09	-	0.00	7.00
7	9.86	9.86	-	9.51	5.02
28	2.50	2.50	-	27.17	7.20
100	0.40	0.40	-	22.85	21.78

* CO_2 is the sum of that from the sediment and the filtered water. Non-extractable residues is the sum of that from sediment and that in the suspended part (filter)

Table A7.1.2.2.2-6 DT_{50} and DT_{90} values for transfluthrin and its degradates in the whole system of flasks incubated in darkness

Compound	**System**	**DT_{50} (days)**	**DT_{90} (days)**	**Quality criterion r^2**
Parent	Laacher Hof	14.8	49.18	0.9918
	Honniger Weiher	7.3	24.2	0.9403

NAK-4452	Laacher Hof	< 14	< 47	estimated
	Honniger Weiher	< 14	< 47	estimated
NAK-4723	Laacher Hof	485	1611	0.9990
	Honniger Weiher	437	1453	0.9998

4.1.4.6 Study 6 - Biodegradation in soil

Report: Reinken, G., Alt, F. and Heruth, D. (2015). Kinetic Evaluation of the Degradation of Transfluthrin and its Metabolite NAK4723 under Aerobic Laboratory Soil Conditions. Bayer CropScience AG, Monheim, Germany. Study No. EnSa-15-0752.

Guidelines: None

GLP No

Test Item: Transfluthrin and 2,3,5,6-tetrafluorobenzoic acid

Material and Methods: A simulation study for soil was performed according to OECD guideline 307, The degradation route and rate of transfluthrin was studied in four different German soils (abbreviated as AX, DD, HH and WW) under laboratory aerobic conditions in the dark at 20±2°C. The test substance was radiolabeled at the methyl-group of the tetrafluorophenyl-moiety of the molecule.

A study application rate of transfluthrin of 45 µg/kg soil dry weight was applied. Due to analytical reasons this corresponds to a dose many times over estimated PECsoil values of transfluthrin resulting from assumed biocidal uses. The test was performed in static systems consisting of Erlenmeyer flasks each containing 50 g soil (dry weight equivalents) and equipped with traps (permeable for oxygen) for the collection of carbon dioxide and volatile organic compounds. Duplicate samples were processed and analysed 0, 0.25, 1, 2, 3, 7 and 14 days after treatment (DAT).

Mean material balances were 87.5% applied radioactivity (AR) for soil AX (range from 82.2 to 100.5% AR), 93.7% AR for soil DD (range from 88.2 to 103.4% AR), 91.6% AR for soil HH (range from 85.2 to 102.2% AR) and 88.3% AR for soil WW (range from 82.5 to 102.7% AR). The maximum amount of carbon dioxide was 68.5, 78.3, 72.5 and 72.9% AR at study end (DAT-14) in soil AX, DD, HH and WW, respectively. Formation of volatile organic compounds (VOC) was insignificant as demonstrated by values of ≤ 0.2% AR at all sampling intervals for all soils. The losses of radioactivity observed throughout the study course were investigated by the Applicant in additional tests to assure that they were not caused by unknown volatile degradation products. Considering their analysis, and the extensively mineralisation of transfluthrin the applicant concluded that the insufficient material balances were caused by losses of carbon dioxide during sample processing.

Non-extractable residues (NER) increased from DAT-0 to DAT-7 from 1.1 to 8.9% AR in soil AX, from 2.3 to 12.1% AR in soil DD, from 1.5 to 10.8% AR in soil HH and from 1.2 to 10.0% AR in soil WW. From DAT-7 to DAT-14 NER slightly decreased to 7.6% AR in soil AX, 10.4% AR in soil DD, 8.9% AR in soil HH and 8.6% AR in soil WW.

Besides the formation of carbon dioxide, one degradation product, i.e. NAK 47234 (2,3,5,6 tetrafluorobenzoic acid, BCS-AA52185), was identified in all investigated soils and with a maximum occurrence of 36.5% AR at DAT-2 in soil HH.

The applicant submitted a kinetic evaluation of this laboratory aerobic degradation (Reinken et al., 2015; Report No.: EnSA-15-0752). The model fit as well as the statistical evaluation of the results were carried out with the software KinGUI, version 2.1. The selection of the most appropriate kinetic model was based on a detailed statistical analysis including visual assessment, $\chi 2$ statistics, randomness of residuals, and t-test significance following the FOCUS guidance (2006, 2014).

Table 1 : Soil degradation not-normalised and normalised (to pF2 and 20°C, Q10: 2.58), DT50 values of transfluthrin for modelling purposes (modelling endpoints) (Reinken et al., 2015)

Transfluthrin	**Dark aerobic conditions**					
Soil type	**pH[a)]**	**t. °C / % MWHC**	**DT50 /DT90 (d)**	**DT50 (d) 20 °C pF2/10kPa[b]**	**St. ($\chi 2$)**	**Method of calculation**

4 Also called TFB-COOH in the consultation

				)		
Sandy loam (Laacher Hof AXXa)	6.0	20±2 / 55	1.282 / 4.26	1.282	7.766	SFO
Clay loam (Dollendorf)	7.3	20±2 / 55	0.934 / 3.10	0.934	7.410	SFO
Silt loam (Hoefchen am Hohenseh)	6.2	20±2 / 55	0.861 / 2.86	0.861	5.319	SFO
Loam (Wurmwiese)	5.1	20±2 / 55	1.057 / 3.51	1.057	6.513	SFO
Geometric mean			**1.022** / 3.39	1.022		

a) Measured in calcium chloride solution

b) Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7

Table 2 : Soil degradation not-normalised and normalised (to pF2 and 20°C, Q10: 2.58) DT50 values (SFO fit) and formation fraction (ff) of NAK 4723 for modelling purposes (modelling endpoints) (Reinken et al., 2015)

NAK 4723	**Dark aerobic conditions Precursor from which the f.f. was derived was transfluthrin**						
Soil type	**pH[a)]**	**t. oC / % MWHC**	**DT50/ DT90 (d)**	**f. f.**	**DT50 (d) 20 °C pF2/10kPa [b)]**	**St. (χ2)**	**Method of calculation**
Sandy loam (Laacher Hof AXXa)	6.0	20±2 / 55	1.495 / 4.97	0.729	1.495	6.288	SFO
Clay loam (Dollendorf)	7.3	20±2 / 55	1.471 / 4.89	0.669	1.471	9.756	SFO
Silt loam (Hoefchen am Hohenseh)	6.2	20±2 / 55	1.433 / 4.76	0.789	1.433	8.966	SFO
Loam (Wurmwiese)	5.1	20±2 / 55	1.317 / 4.38	0.530	1.317	12.614	SFO
Geometric mean			**1.427** / 4.74		1.427		
Arithmetic mean				0.679			

a) Measured in calcium chloride solution

b) Normalised using a Q10 of 2.58 and Walker equation coefficient of 0.7

Evaluation by eCA DE

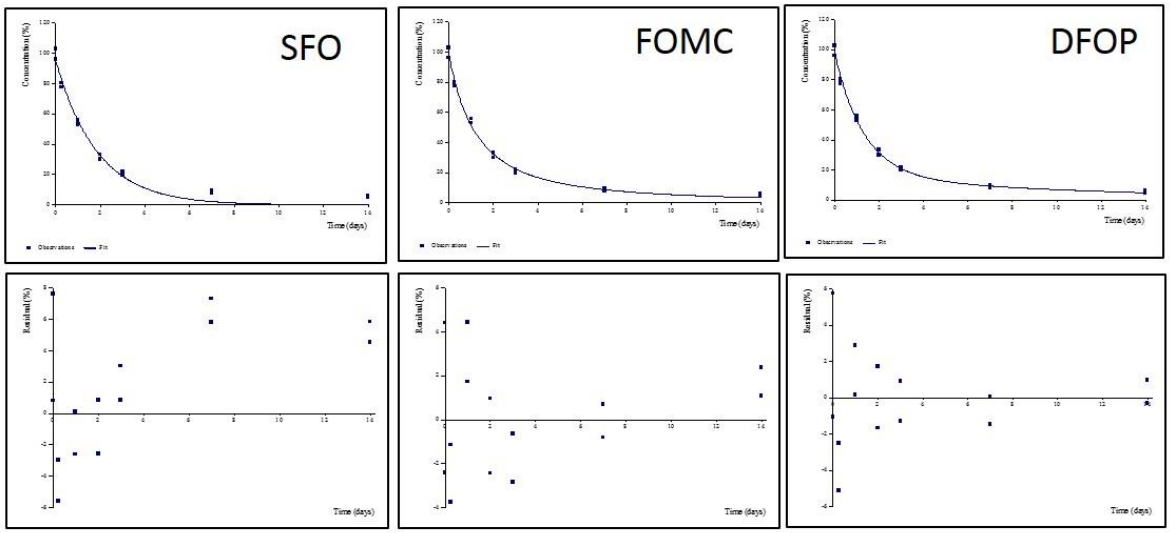
SFO still overestimates degradation in all soils using replicate values, just providing a good fit between day 0 and 3 of incubation. Therefore, in our opinion, SFO fits are not good enough for all soil.

When comparing FOMC with SFO, the FOMC fit from the Höfchen soil study still does not provide an acceptable visual fit, since the two last sampling points are above the fitting curve and residuals are

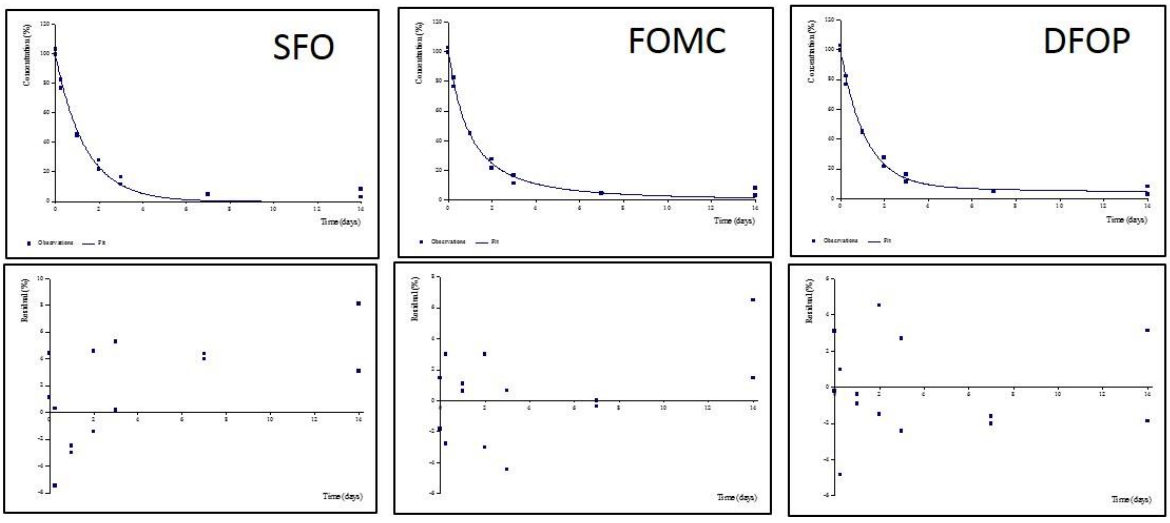
predominately above the zero line. This indicates, in our opinion, a systematic over-prediction of degradation. Where the fit provided by FOMC is considered inadequate, FOCUS allows evaluation of DFOP based on case by case decision[5]. Taking into account the precautionary principle and based on the experiences with other pyrethroids, being all highly adsorptive and showing clear biphasic behavior, we would still suggest that at least for the Höfchen soil DFOP should be applied, resulting in a DT50 of 17.5 days (20°C) derived from k2.

Regarding the soils Laacher Hof, Dollendorf II and Wurmwiese, we think, after recalculation, that the use of the FOMC model could be acceptable. The obtained DT50 values calculated by dividing the DT90 by 3.32 are presented in Table 3.

Laacher Hof

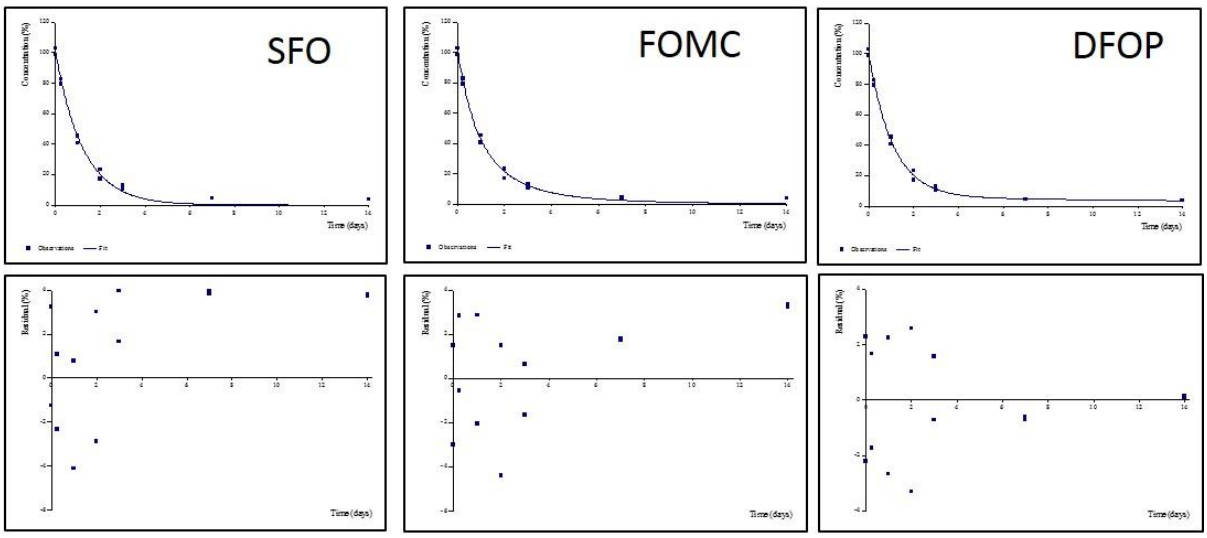


Dollendorf II



5 FOCUS, p. 119: For experiments where concentrations decrease to 10% of the initial value within the study period, half-lives for pesticide fate modelling can be calculated from the DT90 value for the bi-phasic Gustafson and Holden model (FOMC). The DT90 value is divided by 3.32 for conversion to a half-life (for first-order kinetics, the half-life is 3.32 times shorter than the DT90 value). This recalculated half-life is longer than the original half-life and its use as an input for pesticide fate modelling will result in an over-estimation of pesticide residues in soil. The FOMC model was selected as the single standard option in this situation. Hockey-stick and bi-exponential kinetics are not tested at this stage, because these models have a larger number of parameters than the FOMC model. All three models are likely to result in similar DT90 values where measured concentrations decrease to 10% of the initial value within the study period. However, alternative models can be evaluated if the fit by FOMC kinetics is inadequate.

Höfchen



Wurmwiese

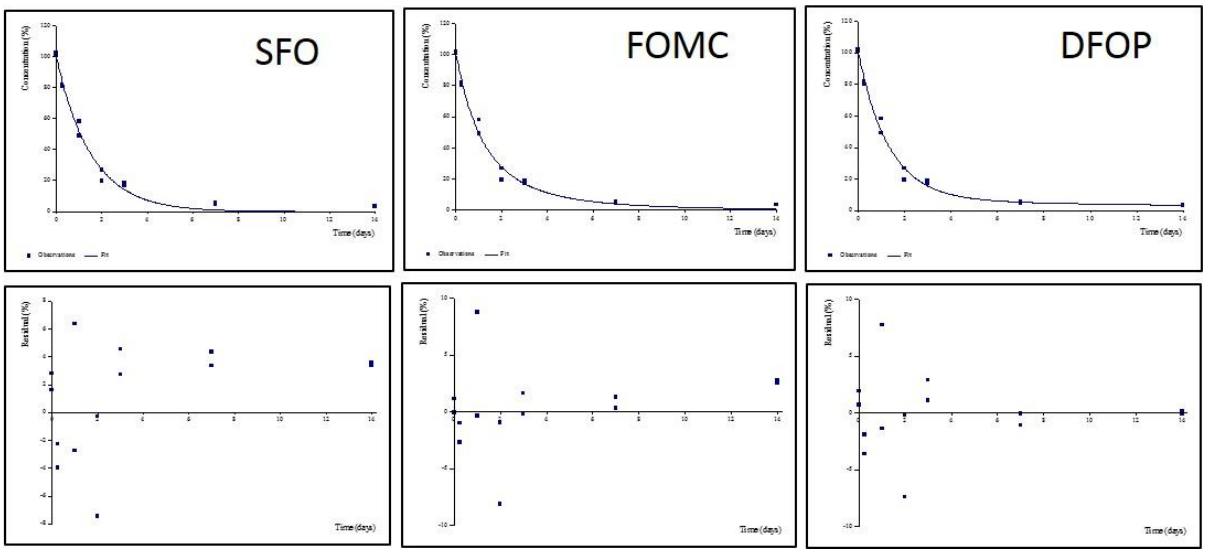


Table 3: Modelling endpoints of transfluthrin and NAK 4723 as well as formation fractions

	Model	χ2	$DT_{50\text{-modelling}}$ (20°C)	$DT_{50\text{-modelling}}$ (12°C)	f.f.
Laacher Hof				Laacher Hof_parent FOMC_metabolite SI	
Parent	FOMC	3.98	1.93	3.66	
NAK 4723	SFO	13.8	1.93	3.66	0.6231

Whole model		7.68			
Dollendorf II				Dollendorfll_parent FOMC_metabolite Sl	
Parent	FOMC	4.38	1.29	2.45	
NAK 4723	SFO	10.5	1.79	3.40	0.6130
Whole model		7.17			
Höfchen				Höfchen_parent DFOP_metabolite SF	
Parent	DFOP	0.87	18.3	34.71	
NAK 4723	SFO	10.1	1.6	3.03	0.7512
Whole model		6.03			
Wurmwiese				Wurmwiese_parent FOMC_metabolite Sl	
Parent	FOMC	6.46	1.28	2.4	
NAK 4723	SFO	16.1	1.53	2.9	0.4886
Whole model		10.0			
DT50 for PEC (geometric mean)			Parent: 2.76 NAK 4723: 1.71	Parent: **5.17** NAK 4723: **3.23**	
f. f. for PEC (arithmetic mean)					**0.6190**

The following DT50 values and formation fractions were concluded for transfluthrin and the metabolite:

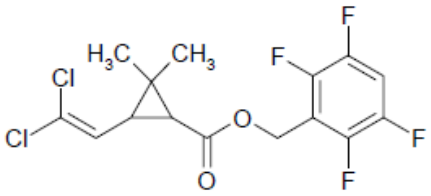
DT50lab (12°C, aerobic) geometric mean from three soils using FOMC and one soil using DFOP: Parent-DT50: 5.17 d (12°C),

Metabolite NAK 4723 (2,3,5,6-tetrafluorobenzoic acid, BCS-AA52185)-DT50: 3.23 d (12°C),

Formation fraction: 0.6190

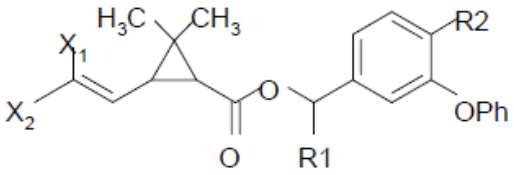
4.1.4.7 Study 7 - Phototransformation in water including identity of transformation products

Document. IIIA/ Section A7.1.1.1.2/03 **BPD Data set IIA/ Annex Point IIA7.6.2.2**	**Phototransformation in water including identity of transformation products**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure []	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [✓]	
Detailed justification:	**Justification for not repeating an aqueous photolysis study on transfluthrin in the frame of the Biocidal Product Directive** **1 Introduction.** Transfluthrin, [(2,3,5,6-tetrafluorophenyl)methyl]1R,3R-(2,2-dichloroethenyl)-2,2-dimethyl cyclo-propanecarboxylate (see Figure 1), is a photo stable Class 1 synthetic pyrethroid developed by Bayer in the 1980s for control of various flying and crawling insects found in and around the home. **Figure 1:**  Due to the fact the majority of transfluthrin containing products sold in Europe historically have been used for indoor application (the exception being a low concentration mosquito coil product); direct exposure of water to transfluthrin has been qualitatively considered very low. Therefore, less information on transfluthrin environmental fate and effects has been generated than for the various synthetic pyrethroids used in field crop protection. Transfluthrin is now regulated in Europe as a Biocidal Product - PT-18, Insecticides, acaricides and products to control other arthropods - under Directive 98/8 EC. In order to be granted re-registration, transfluthrin must be shown to be safe for all exposed environmental compartments. A respective data package in order to risk assess the aqueous environment is available, i.e. on abiotic degradation (hydrolysis, photolysis) and biodegradation in water/sediment system. However, during the evaluation and the peer-review of the dossier, the request for a new aqueous photolysis study was raised by some member states. This paper aims to demonstrate that repeating a new guideline study on direct photo-transformation in water would not add relevant information for the risk assessment. In fact the photodegradation of transfluthrin in water was not yet investigated by a study performed according to currently available test guidelines. However, there exists a significant body of studies on Class 1 and Class 2 synthetic pyrethroid environmental fate and effects within Bayer CropScience, and in the open literature as well. A large proportion	

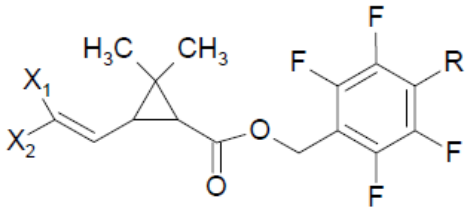
of photo stable synthetic pyrethroids used in crop protection contain a 3-vinyl substituted cyclopropyl acid moiety linked through a central ester bond to a substituted benzyl alcohol moiety. Bayer CropScience is a data owner for 3 synthetic pyrethroids in addition to transfluthrin: cyfluthrin, deltamethrin, and permethrin (see Figure 2). Furthermore, extensive published data is available on several pyrethroids including permethrin and its cyano-analogue cypermethrin as well as on a pyrethroid containing a 3-vinyl substituted cyclopropyl acid moiety linked through a central ester bond to a para-substituted tetrafluorobenzyl (see Figure 3).

Figure 2:



	CAS No.	Xi	x2	Ri	R2
Cyfluthrin	68359-37-5	Cl	Cl	CN	F
Cypermethrin	52315-07-8	Cl	Cl	CN	H
Deltamethrin	52918-63-5	Br	Br	CN	H
Permethrin	52645-53-1	Cl	Cl	H	H

Figure 3:



	CAS No.	Xi	x2	R
Transfluthrin	118712-89-3	Cl	Cl	H
Tefluthrin	79538-32-2	Cl	CF3	CH3

A significant proportion of photo stable synthetic pyrethroids used in crop protection contain a 3-vinyl substituted cyclopropyl acid moiety linked through a central ester bond to a substituted benzyl alcohol moiety. The following sections will demonstrate that for pyrethroids of similar structure to transfluthrin, the degradation pattern is consistent across Class 1 and Class 2 pyrethroids.

2 Hydrolysis.

Abiotic hydrolysis studies provide information on the potential stability of compounds in water at pH values expected in the environment (pH 5, 7, and 9). Under abiotic hydrolysis conditions at 25°C, transfluthrin was found to be stable at pH 5 and 7 (T½ > 1 year) but was degraded by ester cleavage to 2,2',3,3'-tetrafluorobenzyl alcohol and 3-(2,2-dichloroethynyl)-2,2-dimethylcyclo-propane carboxylic acid (a.k.a. permethrinic acid or DCVA) at pH 9 with a half-life of 14 days.

It should be considered that an alkaline pH showing the highest instability of the pyrethroids is most common in surface waters exposed to sunlight. Due to the fact that via photosynthesis of algae and aquatic

plants the dissolved carbonate is consumed during the day the pH is increasing to alkaline pH, always.
Hydrolysis studies on other pyrethroids show similar results (see Table 1 and Table 2).
Conclusion: Based upon the results presented, it can be concluded that Class 1 and Class 2 pyrethroids similar in structure to transfluthrin are susceptible to hydrolytically cleavage of the ester bond under alkaline conditions in water, which are most common in surface water bodies exposed to sunlight.

3 Photolysis.

In an experiment on transfluthrin (Hellpointner, 1991), the UV-absorption spectrum of 4.65 mg transfluthrin per liter of water/acetonitrile[6] 1:1 (v:v) showed an absorption maximum at 210 nm (ε = 18200: band width up to 226 nm) and a secondary maximum at 265 nm (ε = 1277; band width up to 275 nm). The absorption of transfluthrin ended at 279 nm with ε <10 I/mole cm. Therefore, the direct interaction of transfluthrin with the sunlight is not expected to be a relevant route of degradation in water. Even when assuming a theoretical quantum yield of 1.0, not any direct phototransformation is possible in the environment in such a case.
For crop protection products to be registered in the EU or NAFTA currently, this type of study can be waived if the UV-VIS absorption spectra of the test item do not indicate absorption of environmental sunlight (i.e. molar absorption coefficient of $\varepsilon \geq 10$ L mol-1 cm-1 at wavelengths above 295 nm. Exactly that is the case for transfluthrin as described above.
[References: Draft of revised version of Annex II to Council Directive 91/414/EEC Rev.7.0, May 2007). OECD Guideline 316: Phototransformation of Chemicals in Water – Direct Photolysis (adopted October 2008)]
Despite the before-mentioned fact direct phototransformation of transfluthrin was examined historically, e.g. in a non-guideline study (Anderson, 1987) using samples prepared in sterile-filtered water. Samples (in quartz cells) were irradiated using a mercury vapour light source using a carousel apparatus for periods of up to 8 hours. Indirect photolysis in the presence of humic acids was also conducted.
Whenever evaluating results of phototransformation studies with organic compounds, e.g. of pyrethroids (see a compilation of data in and Table 4) the respective purpose and detailed parameters of study (light source, light intensity, cut-off filters of wavelengths, as well as the ingredients and impurities of test solutions) should be considered carefully, always.
Conclusion: Based upon the results presented, it can be concluded that Class 1 and Class 2 pyrethroids similar in structure to transfluthrin are susceptible to cis/trans-photo isomerisation and to ester cleavage leading to the formation of phenoxybenzoic acid, phenoxybenzoic aldehyde, or phenoxybenzoic alcohol and the corresponding vinyl acid under aqueous conditions.
The tests where transfluthrin degraded photolytically with a half life of 17 hours were set up in order to determine the quantum yield of direct

[6] The solubility of tansfluthrin in water was given to be 57 µg/L at 20°C while its solubility in a mixture of water/acetonitrile (99:1) raised up to 100 µg/L

phototransformation, but not to simulate real environmental outdoor sunlight conditions. Experience shows that a DT50 of 17 hours in such equipment is regarded comparatively long, and the product formation might have been rather artificial as well. The other photodegradation tests, i.e. including natural waters or humic acids, did have the purpose to investigate the potential of indirect phototransformation and to identify its degradates, but not testing the direct phototransformation.
A further fact to be considered i.e. for pyrethroids is their commonly very low water solubility and, on the other hand, their high sorption potential to organic matter (indicated by the high Kd or Koc values). If entries into surface water might occur, this leads to a very fast disappearance from the water body to any surfaces. In consequence, the compound will not be accessible for direct phototransformation in water for a longer time period.

4 Conclusion.

For the following reasoning direct photolytic degradation in water is not expected to be a relevant route of degradation of transfluthrin in water:
a) As for a crop protection product to be registered in the EU or NAFTA, this type of study can be waived for a compound like transfluthrin showing not any UV-absorption in the environmentally relevant wavelengths occurring on earth's surface. Such compounds are regarded as stable with respect to direct phototransformation in water.
b) Due to the hydrophobic nature of transfluthrin residues entering into water will rapidly be adsorbed on sediment and transfluthrin will undergo a rapid degradation in a microbial active system (Hellpointner, 1993). Thus, transfluthrin will never be accessible to phototransformation for a longer time period.
c) Transfluthrin indicates lowest hydrolytic stability in alkaline water, which is most prominent whenever natural surface waters are exposed to sunlight.

Table 1: Hydrolysis Results for Synthetic Pyrethroids, Bayer data

Pyrethroid	C°	DT 50	Degradates	Reference
Transfluthrin	25	pH 5, stable pH 7, stable pH 9, 14 days	DCVA; 2,3,5,6-tetrafluorobenzyl alcohol	Hellpointner, 1989
Cyfluthrin	25	pH 5, stable pH 7, 193 days pH 9, <2 days	Presumed: DCVA 4-fluorophenoxy-benzaldehyde	Sandie, 1983
Cyfluthrin	20	pH 5, stable pH 7, 270days diast I+II, 160days diast III+IV pH 9, 42h diast I+II, 33h diast III+IV	assumed as above	Krohn, 1997
Deltamethrin	25	pH 5, stable pH 7, stable pH 9, 2.5 days	3-(2,2-dibromoethynyl)-2,2-dimethylcyclopropane carboxylic acid (Br_2CA); 3-phenoxy-benzaldehyde	Smith, 1990
Permethrin	25	pH 5.7, >200 days pH 7.6, >200 days pH 9.6: 40-60 days	DCVA; 3-phenoxybenzyl alcohol	Alvarez & Dziedzic, 1977 (FMC study, BES access for PT-08)

Table 2: Hydrolysis Results for Synthetic Pyrethroids, published data

Document. IIIA/ Section A7.1.1.1.2/03
BPD Data set IIA/ Annex Point IIA7.6.2.2

Phototransformation in water including identity of transformation products

Pyrethroid	C°	DT 50	Degradates	Reference
Cypermethrin (cis:trans)	25 °C	pH 3, cis, trans <10% hydrol, 28 d pH 7, cis < 10% hydrol, 28 d pH 7, 136 d trans pH 11, 38 min cis pH 11, 23 min trans	(RS)-α-carbamoyl-3-phenoxybenzyl (\R)-cis, trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylate DCVA 3-phenoxybenzaldehyde	Takahashi *et al.* 1985a
Cypermethrin (cis:trans)	25°C	pH 5, stable pH 7, stable pH 9, 1.8-2.5 d	*cis*- and *trans*-DCVA; 3-phenoxybenzaldehyde	Pesticide residues in food 2008, zeta-cypermetrin
Tefluthrin	25°C,	pH 5- pH 7: hydrolytically stable pH 9: > 30 d	3-(2-chloro-3,3,3-trifluoroprop-1-enyl)-2,2-dimethylcyclopropanecarboxylic acid 2,3,5,6-tetrafluoro-4-methylbenzyl methanol	DAR, 2006

Table 3: Aqueous photolysis results for synthetic pyrethroids, Bayer data

Pyrethroid	Conditions	DT_{50}	Degradates	Reference
Transfluthrin	10-30 µg/L in solution of 2% acetonitrile.Mercury vapour lamp. Sodium sulphatebefore GC analysis	17h	Cis-isomer of transfluthrin, 2,3,5,6-tetrafluorobenzyl 3-formyl-2,2-dimethylcyclopropane carboxylate Alkyne form of transfluthrin	Anderson, 1987
Cyfluthrin Phenyl-U-labelled	Solution of 1% acetonitrile, sterile, pH5; natural Kansas summer sunlight; Conc. 5 µg/L	< 1 day	4-fluorophenoxy-benzaldehyde (max. 18%) and -fluorophenoxy-benzoic acid (max. 37%)	Gronberg, 1987
Cyfluthrin Phenyl-U-labelled and fluorophenyl – UL- labelled	Solution of 1% acetonitrile, sterile, pH5; medium pressure; light intensity of 6700 µW/cm^2 ; Conc. 5 µg/L	12.2 days	4-fluorophenoxy-benzaldehyde (max.3% 3-fluorophenoxy-benzoic acid (max. 8.5%)	Puhl et al., 1983
Deltamethrin	Solution of 1% acetonitrile, sterile, pH5; xenon arc light system. Wavelength range was 310 – 740 nm	48 days in the non-sensitised, and 4 days in the sensitised system	mPBacid = 3-phenoxy-benzoic acid (major metabolite) mPBalcohol = 3-phenoxy-benzenemethanol cis-Br_2CA = (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropane carboxylic acid trans-isomer of deltamethrin	Bowman and Carpenter, 1987
Deltamethrin (benzyl labelled deltamethrin gem-dimethyl labelled deltamethrin	in sterile buffer solution; pH 7; 30 µg/l, with 1% acetonitrile	64 days 86 days	mPBacid = 3-phenoxybenzoic acid cis-Br_2CA = (1R-cis)-3-(2,2-dibromoethenyl)-2,2-dimethylcyclopropanecarboxylic acid	Wang and Reynolds, 1991
Permethrin	under sterilised buffer solution at 25°C. Xenon Arc lamp	37.7 days	Up to 19 transformation products were observed, the greatest of which accounted for 5.6%	Amos. and Donelan, 1987 (Syngenta study, BES access for PT-08)

Table 4: Aqueous photolysis results for synthetic pyrethroids, published data

Document. IIIA/ Section A7.1.1.1.2/03 **BPD Data set IIA/ Annex Point IIA7.6.2.2**	**Phototransformation in water including identity of transformation products**

Pyrethroid	Conditions	DT_{50}	Degradates	Reference
Cypermethrin cyclopropyl label, benzyl ring label	Aqueous sterile buffer pH 7, 0.1 mg/L, 25 °C, natural sunlight	36 and 20 days	PBA and DCVA.	Pesticide residuesin food 2008, zeta-cypermethrin
alpha-cypermethrin	in sterile aqueoussolution (0.002 mg/L), pH 5 at 22°C, was exposed to artificial light	*ca.* 48h	*Cis*- and *trans*-DCVA, 3-phenoxybenzoic acid 3-phenoxybenzaldehyde	Pesticide residuesin food 2008, alpha-cypermethrin
Cypermethrin cis:trans 40:60	4 µg/L in sterile pH 4 buffer at 20 °C for 100 hours.	7.1 and 8.9 days	3-phenoxybenzoic acid (15%), 3-phenoxybenzaldehyde (3%) DCVA (18%).	Pesticide residuesin food 2008, cypermethrin
Cypermethrin Cis-trans isomers labelled either in the cyclopropyl, cyano or benzyl ring positions	Distilled water, 2% aqueous acetone, natural river water (pH 8.7), natural seawater (pH 8.3); humic acid (1 ppm) aqueous solution; natural sunlight for 10 days (ca 8 hours per day).	In distilled water: up to 3.6 days In natural water : up to 1 day	3-phenoxybenzoic acid (PBA) 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCVA.) Both cypermethrin isomers were subject to isomerisation of the cyclopropane ring under photolysis conditions	Takahashi et al. 1985b
Tefluthrin Cyclopoipyl and phenyl labelled	25°C, at the solubility limit of tefluthrin – 20µg/L	> 31 days	Isomerization to trans-tefluthrin was the only observed degradate in amount of between 21.2% and 37.2 after 31 days	DAR, 2006

Study References:

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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	*01-08-2013*
Evaluation of applicant's justification	*Information on photolysis in water is not required in order to perform the risk assessment for surface water.*
Conclusion	*Further information is not 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	

4.1.4.8 Study 8 – Phototransfomation in water

Document. IIIA/ Section A7.3.1 BPD Data set IIIA/ Annex Point VII.5	Phototransformation in air (estimation method), including identification of breakdown products

	Reference	**Official use only**
Data protection	Yes	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes Guideline EC Directive 94/37/EC Guideline EC Directive 95/36/EC	
GLP	Not applicable for the modelling report.	
Deviations	None	
	Materials and Methods	
Estimation procedure		
Estimation method	*Based on an estimation according to structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers, the half life time in air of transfluthrin was assessed by the Atmospheric Oxidation Program, AOPWIN (v1.91).* *The Atmospheric Oxidation Program for Microsoft Windows (AOPWIN version 1.9, US EPA) estimates the rate constant for the atmospheric, gas-phase reaction between photochemically produced hydroxyl radicals and organic chemicals. It also estimates the rate constant for the gas-phase reaction between ozone and olefinic/acetylenic compounds. The rate constants estimated by the program are then used to calculate atmospheric half-lives for organic compounds based upon average atmospheric concentrations of hydroxyl radicals and ozone.*	
Test performance	*The accuracy of the estimation methods used by the Atmospheric Oxidation Program has been examined by comparing a list of more than 640 experimentally determined hydroxyl radical rate constants to the program's estimated rate constants. Over 90 percent of the estimated rate constants for the 647 different chemicals are within a factor of two of the experiment value (AOPWIN version 1.9, US EPA).*	
	Results	
Calculations		

	Scenarios, half-lives and chemical lifetimes of transfluthrin in air, as estimated by AOPWIN (v1.91) are detailed in Table A7.3.1-01. the following is the output generated by AOPWIN: SMILES : Fc1c(F)cc(F)c(F)c1COC(=O)C2C(C)(C)C2C=C(CL)CL CHEM : Transfluthrin MOL FOR: C15 H12 CL2 F4 O2 MOLWT:371.16 <u>SUMMARY (AOP vl.91): HYDROXYL RADICALS</u> Hydrogen Abstraction = 1.9059 E-12 cm3/molecule-sec Reaction with N, S and -OH = 0.0000 E-12 cm3/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Olefinic Bonds = 3.8323 E-12 cm3/molecule-sec **Addition to Aromatic Rings = 0.8661 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 6.6043 E-12 cm3/molecule-sec HALF-LIFE = 2.429 Days = 58.304 Hrs (24-hr day; 0.5E6 OH/cm3) HALF-LIFE = 1.620 Days = 19.435 Hrs (12-hr day; 1.5E6 OH/cm3) ** Designates Estimation(s) Using ASSUMED Value(s) <u>SUMMARY (AOP vl.91): OZONE REACTION</u> OVERALL OZONE Rate Constant = 0.023261 E-17 cm3/molecule-sec HALF-LIFE = 49.268 Days (at 7E11 mol/cm3) Experimental Database: NO Structure Matches
Degradation product(s)	*Not determined by this theoretical estimation method. However this is a standard and widely accepted method.*
	Applicant's Summary and conclusion
Materials and methods	*The Atmospheric Oxidation Program for Microsoft Windows (AOPWIN version 1.9, US EPA), based upon the structure-activity relationship (SAR) methods developed by Dr. Roger Atkinson and co-workers, was used to estimate the rate constant for the atmospheric, gas-phase reaction between transfluthrin and photochemically produced hydroxyl radicals and with ozone. The estimated rate constants are then used within the program to calculate the atmospheric half-lives based upon average atmospheric concentrations of hydroxyl radicals and ozone.*
Results and discussion	The estimated atmospheric half-life of transfluthrin for gas-phase reactions with photochemically produced hydroxyl radicals ranged between 19.4 hours (equivalent to 1.6 days, based upon typical OH radical concentration during daylight hours) and 58.3 hours (equivalent to 2.4 days, based upon typical OH radical concentration averaged over day and night times). It should be noted that the OH radicals are very reactive after being generated due to irradiation of certain atmospheric constituents by the sunlight. In reality, their concentration is 0 during the night. Therefore, the scenario with 1.5 x 10^6 radicals cm^{-3} during the daytime (and a corresponding concentration of 0 during the night) and a tropospheric half life of 1.6 days for transfluthrin is the more realistic

	estimation. This scenario is also favoured by the US Environment Protection Agency mentioned in the legend of AOPWIN. Ozone reactions can also contribute to the disappearance of transfluthrin in the troposphere, but at a minor extent, only ($t_{½}$ = 49 days at 7 x 10^{11} mol ozone cm^{-3}). Results are summarised in Table 7.3.1-01. Transfluthrin may be expected to be highly susceptible for reactions with hydroxyl radicals, which will contribute significantly to the overall degradation of the substance in the atmosphere. Various moieties of the molecule were identified as possible targets for radical reactions. Attack by hydroxyl radicals should result in the formation of multiple primary radicals. These may lead to secondary oxidation products, which can be eliminated from the air by wet and/or dry deposition. The degradation by ozone is possible, but to a minor extent. Reactions with other reactive species and direct gasphase or liquid-phase photolysis are not considered in the employed model calculation, but will also contribute to the overall atmospheric elimination of transfluthrin.
Conclusion	*From the short half-life time in air, it is to be expected that transfluthrin cannot be transported in gaseous phase over large distances and cannot accumulate in the atmosphere.*
Reliability	*1*
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	*23-03-2007*
Materials and Methods	*Applicant's version adequately reflects the report.*
Results and discussion	*Applicant's version is adopted*
Conclusion	*Applicant's version is adopted. Half-life = 2.429 days (24-hr day; 0.5E6 OH/cm3); Half-life = 1.620 days (12-hr day; 1.5E6 OH/cm3)]. The atmospheric half-life of 2.4 days (calculated conform TGD) will be used for risk assessment.*
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 7.3.1-01: Scenarios, half-lives and chemical lifetimes of transfluthrin in air, as estimated by AOPWIN (v1.9.1.)

Scenario of OH concentration used		**Long term**	**Short term**
Time frame	[hours/day]	24	12
OH concentration	[radicals/cm^3]	0.5 x 10^6	1.5 x 10^6
OH rate constant	[cm^3 x $molecule^{-1}$ x s^{-1}]	6.6043 x 10^{-12}	
Half life (t½) due to reaction with OH	[hours] [days]	58.3 2.4	19.4 1.6
Chemical lifetime (τ) due to reaction with OH	[hours] [days]	84.1 3.5	28.0 2.3
Ozone concentration	[mol cm^{-3}]	7 x 10^{11}	
Ozone rate constant	[cm^3 x $molecule^{-1}$ x s^{-1}]	0.023261 x 10^{-17}	
Half life (t½) due to reaction with ozone	[days]	49	

4.1.4.9 Study 9 - Field soil dissipation and accumulation

Document IIIA/Section 7.2.2.2 **BPD Data Set IIIA/ Annex Point XII.1.1**	**Field soil dissipation and accumulation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	Refer to 7.2.1 for detailed justification. A detailed justification for the non-submission of further aerobic soil studies has been presented within section 7.2.1 based upon the negligible exposure to the terrestrial compartment (PECsoil of 6.8 x 10^{-11} mg/kg) and the fate of transfluthrin being confidently predicted by the fate documented for other pyrethroids of comparative structure and function. It is predicted that under normal agricultural practice, transfluthrin will not persist or accumulation in the terrestrial compartment. Therefore the need to conduct further soil dissipation and accumulation studies is considered to be scientifically unjustified.	
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*23-03-2007*	
Evaluation of applicant's justification	*The statement that the fate of transfluthrin can be confidently predicted from that of other pyrethroids is not agreed upon (see Doc III 7.2.1). However, for the proposed uses of transfluthrin, direct emission to soil is considered negligible. Since no risks for the terrestrial compartment are identified as a result of indirect emissions, it is not considered necessary to perform additional studies.*	
Conclusion	*Further information is not required.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	
Date		
Evaluation of applicant's justification		
Conclusion		

Document IIIA/Section 7.2.2.2 **BPD Data Set IIIA/ Annex Point XII.1.1**	**Field soil dissipation and accumulation**
Remarks	

4.1.4.10 Study 10 – Aerobic aquatic degradation

Document IIIA/Section 7.1.2.2.1 **BPD Data Set IIIA/ Annex Point XI.2.1**	**Aerobic aquatic degradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	The biotic degradation of transfluthrin in aquatic systems has been thoroughly evaluated in a water/sediment study (Hellpointner, (1993) – See Doc IIIA, Section 7.1.2.2.2). This study demonstrated the rapid removal and degradation of transfluthrin under aerobic-aquatic conditions (DT_{50} (whole system, dark): 7 – 15 days). Further confirmatory data from indirect aquatic photolysis (See Doc IIIA Section 7.1.1.1.2) demonstrated that in the presence of photosensitisers (humic acid), transfluthrin degrades rapidly in the aquatic environment. The amateur indoor use of transfluthrin (from use of Raid Portable Electric (highest exposures), with subsequent deposition and transference of residues from room surfaces to wastewater, results in negligible concentrations in STP (8.8×10^{-10} – 8.8×10^{-7} mg/l) and surface water (1.9×10^{-11} – 8.2×10^{-8} mg/l). In fact the relatively high log Kow (5.46) and low water solubility (0.0575 mg/L) mean that it is unlikely that transfluthrin will be present in natural water (Doc IIB, section 3.3). Due to limited exposure to aquatic environmental compartments and the biotic degradation demonstrated in the water sediment study the need to conduct studies on the aerobic aquatic degradation in aquatic systems is considered to be scientifically unjustified.	X
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*11-02-2007*	
Evaluation of applicant's justification	*Applicant's statement on photolysis is not considered justified because the photolysis studies are not considered reliable. However, in view of the presence of acceptable data on degradation in water/sediment systems, further studies are not necessary.*	
Conclusion	*No further information needed.*	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

4.1.4.11 Study 11 – Anaerobic biodegradation

Document IIIA/Section 7.1.2.1.2 **BPD Data Set IIIA/ Annex Point XI.2.1**	**Anaerobic biodegradation**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	Exposure to anaerobic conditions through the use of transfluthrin based products is not expected. The amateur indoor use of transfluthrin (from use of Raid Portable Electric (highest exposures), with subsequent deposition and transference of residues from room surfaces to wastewater, results in negligible concentrations in STP (8.8×10^{-10} – 8.8×10^{-7} mg/l) and surface water (1.9×10^{-11} – 8.2×10^{-8} mg/l). In fact the relatively high log Kow (5.46) and low water solubility (0.0575 mg/L) mean that it is unlikely that transfluthrin will be present in wastewater (Doc IIB, section 3.3). Biotic degradation of transfluthrin has been observed in a water-sediment study (Section 7.1.2.2) with a half life in the total system of 7-15 days (incubated in the dark) and also in an aqueous photolysis study, when the sensitizer humic acid was used. Therefore, any transfluthrin released into the aquatic environment will be subsequently degraded, at least under aerobic conditions. Exposure under anaerobic conditions, possibly caused due to leaching into deeper anaerobic soil layers is excluded due to the immobility in soil (high Koc values). Due to limited exposure to aquatic environmental compartments and the biotic degradation demonstrated in the water sediment study, the need to conduct studies on the anaerobic biodegradation of transfluthrin is considered to be scientifically unjustified.	
Undertaking of intended data submission [ ]	*Not applicable*	
	Evaluation by Competent Authorities	
Date Evaluation of applicant's justification Conclusion Remarks	EVALUATION BY RAPPORTEUR MEMBER STATE *11-02-2007* *Applicant's justification is accepted.* *No additional data needed.*	
Date Evaluation of applicant's justification Conclusion Remarks	COMMENTS FROM OTHER MEMBER STATE ***(specify)***	

4.2 Bioaccumulation

4.2.1 Bioaccumulation test on fish

4.2.1.1 Study 1 – Bioconcentration in aquatic organisms

Document IIIA/ Section A7.4.2 **BPD Data Set IIA / Annex Point VII.7.5**	**Bioconcentration in aquatic organisms**

	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	*Yes* OECD 305 (1996) EPA-FIFRA § 72-6 (1982) EPA-FIFRA § 165-4 (1982) OPPTS 850.1730 (1996)	
GLP	*Yes*	
Deviations	None Materials and Methods	
Test material	[Methylene-^{14}C]- Transfluthrin	
Lot/Batch number	Sample ID: BECH 1738	X
Specification	*As given in section 2 of Doc IIIA.*	X
Purity	Radiochemical purity: > 99% (HPLC, TLC) Chemical purity: > 99% (HPLC, UV)	
Further relevant properties	Specific radioactivity: 3.67 MBq/mg Water solubility: 57 µg/L log partition coefficient octanol/water: 5.46	
Radiolabelling	*[Methylene-^{14}C]*	
Method of analysis	*HPLC and LSC*	
Reference substance	*No*	
Method of analysis for reference substance	*Not applicable.*	
Testing/estimation procedure		
Test system/ performance	The study was performed in 2 parts; Part 1 was a 42-day phase to examine the bioconcentration and depuration of [methylene-^{14}C]-Transfluthrin by bluegill sunfish *(Lepomis macrochirus)*, and Part 2 was a 7 to 14-day exposure to investigate the biotransformation of [methylene-^{14}C]-Transfluthrin in fish. <u>Part 1 (bioconcentration):</u> Groups of 60 young bluegill sunfish were exposed in glass aquaria (100 L) under flow-through conditions to nominal concentrations of 0 (DMF solvent control) 65 and 198 ng [^{14}C]-Transfluthrin/L for a period of 28 days (these levels were determined based on the results of previously conducted fish toxicity tests and on the	

detection limit in water). On day 28 of the exposure period the application of [^{14}C]- Transfluthrin was stopped. The depuration phase was initiated (the aquaria were drained to a water height of ca. 5 cm, and then filled with uncontaminated dilution water); the fish were then exposed to flowing, uncontaminated, dilution water for further 14 days. The test aquaria were maintained at a mean water temperature of 22.0-22.2°C. The mean body wet weight of the fish at the beginning of the test was 1.1 ± 0.25 g (Mean ± S.D.), the mean body length was 4.2 ± 0.16 cm (Mean ± S.D.). The initial loading of 0.66 g fish/L and 0.11 g fish/L/day was in accordance with ASTM standard guidance.
The fish were observed initially and every 24 hours on working days for mortality and/or adverse behaviour. Fish were sampled during the exposure period on days 2, 3, 7, 10, 14, 21 and 28 and during the depuration period on days 29, 31 35, 38 and 42. On these days, four fish from each chamber were collected and processed (dissected) individually for *radioassay (LSC). Radioactivity in water samples was measured during the same intervals. On days 0, 28 and 42 of the study four additional fish were taken from each aquarium to determine the lipid content (using a modified method based on Bligh & Dyer (1959)).

(*Analysis of Fish samples on days 2, 3, 7 and 10 showed inconsistencies therefore the method of fish sample preparation was changed for remaining fish to minimize inconsistencies based on the assumed volatility of the test item, which did not allow using the standard procedures for processing of fish samples).

Part 2 (biotransformation): Groups of 15 (30 in total) bluegill sunfish were exposed in glass aquaria (100 L) under flow-through conditions to a nominal concentration of 132 ng [^{14}C]- Transfluthrin/L for a period of 7 and 14 days. The test aquaria were maintained at a mean water temperature of 22.0-22.2°C. The mean body wet weight of the fish at the beginning of the test was 18.2 ± 5.76 g (Mean ± S.D.), the mean body length was 10.5 ± 0.85 cm (Mean ± S.D.). The initial loading was 0.91 g/fish/L/day.
The fish were observed initially and every 24 hours on working days for mortality and/or adverse behaviour. Fish were sampled and divided into edible and viscera tissues on days 7 and 14; on each sampling day fifteen fish were collected and processed (dissected) individually for radioassay (LSC and combustion analysis).
Water samples (3 x 10 ml) were taken from each aquarium during the same intervals and analysed.

For the determination of metabolites in water samples 500 ml were taken from the high concentration level of part 1 (198 ng/L) and from part 2 (132 ng/L). The samples were deep-frozen up to the analysis by HPLC. In addition stock solutions (all concentrations) and water samples were analysed for content and stability in the beginning and the end of the exposure period (by HPLC).

Stock solutions of test material (in 2 L brown glass bottles) were

	prepared using dimethylformamide (DMF) as solvent. Stock solutions at concentrations of 0.65 mg/L, 1.98 mg/L and 1.32 mg/L were prepared to achieve test dose levels of 65 ng/L, 198 ng/L and 132 ng/L (nominal) respectively. 100 µl DMF/L dilution water (= 0.01 vol.-%) were used in this study as solvent carrier. Stock solutions and dilution water were supplied to the test aquaria at a rate of 2.5 mL/h and 25 L/hour respectively. The control aquarium also received an amount of dimethylformamide, which was equivalent to the exposure aquaria. A dosing system was used to maintain mean water concentrations; a ProMinentR mikro g/5a dispenser (for dosing of stock solutions) and flow-meters (for water flow control) were used for the introduction of [14C]-Transfluthrin and diluent water in 2000 ml-mixing cells. The mixture was running continuously into the 100 L test aquaria. The diluter system was calibrated by volumetric measurements of dispenser aliquots and the flow-rate of flow meters. Water quality parameters of dissolved oxygen; temperature and pH were measured initially and throughout the study in each aquarium once a week. In addition, the daily temperature-fluctuation was measured continuously in the control tank and recorded as hourly mean values. TOC was measured at the beginning of the test and then once a week. *For further details of dilution water, test organisms, test system and test conditions see tables A7_4_2-2 to A7_4_2-5.*	
Estimation of bioconcentration	*As experimental data are available no estimates of BCF are required.*	
	Results	
Experimental data		
Mortality/behaviour	The fish showed no mortalities or abnormal behaviour throughout the study in all test vessels.	
Lipid content	The mean lipid content (day 0-28) for the fish used in the study was calculated to be 6.95 % (see table A7_4_2-6).	
Concentrations of test material during test	*During the 28-day bioconcentration (uptake) phase water concentrations ranged from 61.9 ng/L to 70.0 ng/L for the nominal concentration of 65.0 ng/L and from 171 ng/L to 191 ng/L for the nominal concentration of 198 ng/L. The average water concentration (using the mean value for each sample) during the uptake phase was 66.2 ng/L (standard deviation: 2.26) ng/L for the nominal concentration of 65.0 ng/L and 178 ng/L (standard deviation: 7.44) ng/L for the nominal concentration of 198 ng/l. The parent compound Transfluthrin accounted for ca. 67 % to 100 % of the radioactivity in the profiles of water samples.*	X
	During the 7 and 14 day biotransformation phase water concentrations ranged from 102 ng/L to 126 ng/L for the nominal concentration of 132 ng/L. The average water concentration (using the mean value for each sample) was 114 ng/L (standard deviation: 8.14) for the nominal concentration of 132 ng/L. The parent compound Transfluthrin accounted for ca. 79 % to 84 % of the radioactivity in the profiles of water samples.	X
	The analysis of stock solutions of the test compound from all tests revealed that [Methylene-^{14}C] - Transfluthrin was completely stable in stock solutions in the time range of up to 28 days. Characterisation of the radioactivity (based on TRR) in fish sampled	

during 28 days of constant exposure and 14 days of depuration is summarized below:

Conc. (ng/L)	Sampling day	Range of mean values (dpm/g dry weight)		
		Edible parts	Viscera	Whole fish
Control	2 -10	4670.5 - 7767.8	8624.9 - 12777.4	6584.8 - 9978.9
65	2 -10	10683.5 - 15425.1	47260.3 - 66852.9	27952.2 - 38785.2
198	2 -10	18479.2 - 53735.6	92325.0 - 226238.6	52889.9 - 139070.7

Conc. (ng/L)	Sampling day	Range of mean values (dpm/g fresh weight)		
		Edible parts	Viscera	Whole fish
Control	2 –10	1130.1- 1767.1	2650.0 – 3610.6	1773.1 – 2493.5
	14 - 42	165.6 – 365.6	314.2 – 549.7	218.0 – 436.3
65	2 –10	2280.9 – 3492.2	13442.2 – 18700.0	6775.6 – 9629.6
	14 - 42	1500.3 – 9938.5	7265.1 – 59086.6	3732.9 – 28302.4
198	2 –10	3939.2 – 18415.8	24998.4 – 108513.9	12545.4 – 55276.0
	14 - 42	3696.6 – 27601.7	16297.1 – 180594.7	8460.1 – 85493.5

The mean tissue residues (given in µg/kg fresh weight) at steady state are displayed in table A7_4_2-7.

Characterisation of the radioactivity in fish sampled during the biotransformation phase showed that between 74 % and 96 % of the total radioactive residue (TRR) in edibles and viscera could be identified. The major component detected in all fish samples was the active ingredient, transfluthrin. It accounted for ca. 94 % of the TRR in edibles and for 66 % – 82 % of the TRR in viscera. Total radioactive residues (TRR) measured in edibles were 0.030 µg/g (day 7) and 0.037 µg/g (day 14). In viscera, 0.165 µg/g (day 7) and 0.220 µg/g (day 14) were found.

Bioconcentration factor (BCF)

A BCF calculation based on parent compound was not possible, because neither in fish nor in the water phase stable steady state metabolite concentrations were measurable. Therefore all BCF calculations are based on TRR only.

The steady-state-BCF_{TRR} (based on whole fish, wet weight) in the 65 ng/L test level is about 1704 and in the 198 ng/L test level about 1861. The steady-state-BCF_{TRR} normalised to 6% lipid content in fish is in the 65 ng/L test level about 1471 and in the 198 ng/L test level about 1607 (see table A7_4_2-9). X X

Uptake and depuration rate constants

Because of inconsistencies in radioactivity measurements during the uptake phase the uptake rate constant (Ku) could not be calculated.

Depuration rate constant = 0.182 –0.227 $days^{-1}$ (for the 65.0 & 198 ng/L

	exposure groups, respectively). See table A7_4_2-8. (The depuration rate constant (Kd) was determined using the OriginTM non-linear kinetic modeling computer programme).	
Depuration time	DT_{50} = 3.1 – 3.8 days (for the 198 & 65.0 ng/L exposure groups, respectively). See table A7_4_2-8. (The DT50 was determined OriginTM non-linear kinetic modeling computer programme).	
Metabolites	In the water samples collected during the exposure phase not only Transfluthrin, but also its metabolite, Tetrafluorobenzyl alcohol was detected between 9.41 % and 33.4 %. Since Tetrafluorobenzyl alcohol was not present at the beginning (day 0) of the exposure phase but appeared soon after introduction of fish (measured at day 2) to 9.41% and increased to 33.4% on day 28 its appearance is obviously due to metabolic degradation in fish. In the water samples collected during the biotransformation phase Tetrafluorobenzyl alcohol was detected between 15.72 % and 20.83 %. The metabolite Tetrafluorobenzyl alcohol was also detected in edible fish parts between 1.83% and 2.18%. This metabolite was also found in profiles from viscera (1.07 % and 2.87 %) together with its oxidation product, Tetrafluorobenzoic acid (1.95 % and 4.62 %).	*X*
Other Observations	Dissolved oxygen, pH-values and test temperature were measured once a week throughout the study in all aquaria. The dissolved oxygen concentrations ranged between 82 % and 104 % saturation (mean: 93-97 %). The pH values ranged from 7.0 to 7.2 with a mean value of 7.1 for all aquaria. The weekly measured water temperatures ranged between 21.5°C and 22.7°C (mean: 22.0°C - 22.2°C). Moreover the measured temperature inside the control aquarium remained between 21.0°C and 23.0°C (mean: 22.4). Contents of TOC (total organic carbon) in each aquarium were measured once a week. Throughout the whole biological part of the study, all measured TOC values in the test vessels did not exceed the concentration of organic carbon originating from the test item and from the solubilising agent (nominal sum TOC is about 46.3 mg/L for all test levels including control) by more than 10 mg/L as expected by OECD guideline 305.	
Estimation of bioconcentration	*As experimental data are available no estimates of BCF are required.*	
	Applicant's Summary and conclusion	
Materials and methods	The bioconcentration and biotransformation of [methylene-^{14}C]-Transfluthrin was investigated in Bluegill sunfish. The study was conducted in accordance to the EPA Pesticide Assessment Guidelines, Subdivision E, §72-6, Subdivision N, §165-4, OPPTS 850.1730 (1996) and OECD Guideline 305. In the first part of the study (bioconcentration) groups of 60 bluegill sunfish were exposed in glass aquaria (100 L) under flow-through conditions to nominal concentrations of 0 (DMF solvent control) 65 and 198 ng [^{14}C]- Transfluthrin/L for a period of 28 days (these levels were determined based on the results of previously conducted fish toxicity tests and on the detection limit in water). On day 28 of the exposure period the application of [^{14}C]- Transfluthrin was stopped and the depuration phase was initiated. The fish were exposed to flowing,	

uncontaminated dilution water for further 14 days. The test aquaria were maintained at a mean water temperature of 22.0-22.2°C.
The fish were observed initially and every 24 hours on working days for mortality and/or adverse behaviour. Fish were sampled during the exposure period on days 2, 3, 7, 10, 14, 21 and 28 and during the depuration period on days 29, 31 35, 38 and 42. On these days, four fish from each chamber were collected and processed (dissected) individually for *radioassay (LSC). Radioactivity in water samples was measured during the same intervals. On days 0, 28 and 42 of the study four additional fish were taken from each aquarium to determine the lipid content (using a modified method based on Bligh & Dyer (1959)).
(*Analysis of Fish samples on days 2, 3, 7 and 10 showed inconsistencies therefore the method of fish sample preparation was changed for remaining fish to minimize inconsistencies based on the assumed volatility of the test item, which did not allow using the standard procedures for processing of fish samples).

In the second part of the study (biotransformation) groups of 15 (30 in total) bluegill sunfish were exposed in glass aquaria (100 L) under flow-through conditions to a nominal concentration of 132 ng [^{14}C]-Transfluthrin/L for a period of 7 and 14 days. The test aquaria were maintained at a mean water temperature of 22.0-22.2°C.
The fish were observed initially and every 24 hours on working days for mortality and/or adverse behaviour. Fish were sampled on days 7 and 14; on each sampling day fifteen fish were collected and processed (dissected) individually for radioassay (LSC and combustion analysis). Water samples (3 x 10 ml) were taken from each aquarium during the same intervals and analysed.

Metabolite quantification was also carried out; for the determination of metabolites in water samples 500 ml were taken from the high concentration level of part 1 (198 ng/L) and from part 2 (132 ng/L). The samples were deep-frozen up to the analysis by HPLC. In addition stock solutions (all concentrations) and water samples were analysed for content and stability in the beginning and the end of the exposure period (by HPLC).
Stock solutions of test material (in 2 L brown glass bottles) were prepared using dimethylformamide (DMF) as solvent. Stock solutions at concentrations of 0.65 mg/L, 1.98 mg/L and 1.32 mg/L were prepared to achieve test dose levels of 65 ng/L, 198 ng/L and 132 ng/L (nominal) respectively. 100 µl DMF/L dilution water (= 0.01 vol.-%) were used in this study as solvent carrier. Stock solutions and dilution water were supplied to the test aquaria at a rate of 2.5 mL/h and 25 L/hour respectively. The control aquarium also received an amount of dimethylformamide, which was equivalent to the exposure aquaria.
A dosing system was used to maintain mean water concentrations; a ProMinentR mikro g/5a dispenser (for dosing of stock solutions) and flow-meters (for water flow control) were used for the introduction of [14C]-Transfluthrin and diluent water in 2000 ml-mixing cells. The mixture was running continuously into the 100 L test aquaria. The diluter system was calibrated by volumetric measurements of dispenser aliquots and the flow-rate of flow meters.

	Water quality parameters of dissolved oxygen; temperature and pH were measured initially and throughout the study in each aquarium once a week. In addition, the daily temperature-fluctuation was measured continuously in the control tank and recorded as hourly mean values. TOC was measured at the beginning of the test and then once a week.
Results and discussion	During exposure fish exhibited normal behaviour. No mortalities were reported at any treatment level (including the controls) throughout the study. The mean lipid content (day 0-28) for the fish used in the study was calculated to be 6.95 %. The parent compound transfluthrin accounted for 67 % to 100 % of the radioactivity in the profiles of water samples. The metabolite Tetrafluorobenzyl alcohol was detected between 9.41 % and 33.4 %; its appearance is considered to be due to metabolic degradation in fish. The major component detected in all fish samples was the active ingredient, transfluthrin. It accounted for ca. 94 % of the TRR in edible tissues and for 66 % – 82 % of the TRR in viscera. The metabolite Tetrafluorobenzyl alcohol was detected in edible parts between 1.83% and 2.18%. This metabolite was also found in profiles from viscera (1.07 % and 2.87 %) together with its oxidation product, Tetrafluorobenzoic acid (1.95 % and 4.62 %). Transfluthrin has been shown to accumulate in bluegill sunfish with a total residue bioconcentration factor of about 1704 to 1861 X for whole fish (sum of radiolabelled compounds, Transfluthrin parent, metabolites and mineralization products). When exposure ceases, the residues are depurated with a half-life of 3.1 – 3.8 days. After 14 days in uncontaminated water 86 % (nominal concentration of 65.0 ng/L) and 89 % (nominal concentration of 198 ng/L), respectively, of the mean plateau radioactivity were depurated from whole fish. By extrapolation it can be calculated that 95% of the mean plateau radioactivity would have been depurated from whole fish after 13 – 17 days. Due to inconsistencies in radioactivity measurements during the uptake phase the uptake rate constant (Ku) could not be calculated. However the depuration rate constant was calculated to be (whole fish) 0.182 –0.227 $days^{-1}$ (for the 65.0 & 198 ng/L exposure groups, respectively). The average steady-state (days 14 - 28) bioconcentration factors were 612 X (edible parts) and 1704 X (whole fish) for 65.0 ng [^{14}C]-Transfluthrin/L and 640 X (edible parts) and 1861 X (whole fish) for 198 ng [^{14}C]-Transfluthrin/L. These values correspond to the calculated steady-state total residue levels of 40.4 µg/kg edible parts and 113 µg/kg whole fish for 65 ng [14C]- Transfluthrin/L and of 114 µg/kg edible parts and 332 µg/kg whole fish for 198 ng [14C]- Transfluthrin /L, respectively. *A BCF calculation based on parent compound was not possible, because neither in fish nor in the water phase stable steady state metabolite concentrations were measurable. Therefore all BCF calculations are based on TRR only. The steady-state-BCF_{TRR} (based on whole fish, wet weight) in the 65 ng/L test level is about 1704 and in the 198 ng/L test level about 1861. The steady-state-BCF_{TRR} normalised to 6% lipid content in fish is in the 65 ng/L test level about 1471 and in the 198 ng/L test level about 1607.* *Water quality measurements were within acceptable limits.*

Document IIIA/ Section A7.4.2 BPD Data Set IIA / Annex Point VII.7.5	**Bioconcentration in aquatic organisms**

Conclusion	*Continuous exposure over a 28-day period of [methylene-^{14}C]-Transfluthrin to bluegill sunfish resulted in steady-state-BCF_{TRR} (normalised to 6% lipid content in fish) values of 1471 and 1607 at 65 ng/L and 198 ng/L respectively.*
Reliability	*1*
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	*02-03-2007*
Materials and Methods	*Applicant's version is adopted with the following comment:* *3.1.1/3.1.2 Batch differs from those included in the batch analysis (Doc III A.2 confidential), but purity of test substance is adequate.*

Results and discussion

Applicant's version is adopted with the following additions/amendments:

4.1.3 At exposure concentration 198 ng/L (bioconcentration study), concentrations of transfluthrin in the water phase decreased with time from 100 % of TRR at t=0 to 90.6 % at t=2 and 66.6 % at t=28. Concentrations of TFB-OH increased concurrently from n,d, at t=0 to 9.4 % of TRR at t=2 and 33.4 % at t=28.

At 132 ng/L (biotransformation study), transfluthrin accounted for 79.2 % of TRR at t=7 and 84.3 % at t=14, corresponding values for TFB-OH were 20.8 and 15.7 % of TRR.

No explanation is given for the fact that in the biotransformation study at 132 ng/L concentrations of transfluthrin are stable, this in contrast to the bioconcentration experiment at 198 ng/L.

4.1.4 BCF-values are estimated on the basis of TRR, because parent and metabolite concentrations in water and fish were not stable during the BCF-study. Based on TRR a steady state was reached. However, in the biotransformation part, concentrations of transfluthrin in water and fish were relatively stable. The following data are taken from the Appendix with HPLC analyses (bold figures calculated by RMS):

day	concentration of transfluthrin in			
	water	fish		
		edible	viscera	total
	ug/L	ug/g	ug/g	ug/g
7	0.085	0.028	0.135	**0.163**
14	0.092	0.035	0.145	**0.180**
mean	**0.089**			**0.172**

From this, a BCF of 1938 L/kg is calculated. This figure is in good agreement with the BCF's based on TRR (1704 and 1861 L/kg at 65 and 198 ng/L). Although the BCF of 1938 L/kg is less reliable because it is based on two time points only, the calculation indicates that the BCF based on TRR can be used as a reliable estimate of the BCF for transfluthrin.

The BCF is normalised to a lipid content of 6 %. It should be noted that a lipid fraction of 5 % is currently proposed within the framework of REACH[7]. Using this fraction, normalised BCF-values would be 1226 and 1339 L/kg at 65 and 198 ng/L, respectively. For the present assessment, however, normalisation is not applied, since lipid content (6.95%) was not outside the normal range.

4.1.6 The depuration time (DT_{90}) is 10.1-12.7 days

4.1.7 The conclusion that the presence of metabolite tetrafluorobenzyl alcohol is due to biotransformation in the fish and subsequent excretion of metabolites to the water is not necessarily true. The presence of metabolites in the water phase may also be due to degradation by bacteria in the water. This observation, however, does not influence the results.

The level of metabolites (%) in organisms accounting for > 10% of residues is for TFB-OH and TFB-COOH < 5%.

Conclusion

Applicant's version is adopted.
BCF-values of 1704 and 1861 L/kg are used for risk assessment.

Reliability

2
Instability of the test substance, BCF value based on TRR

Acceptability

acceptable

[7] DRAFT RIP 3.3-2 EWG 10 Aquatic Bioaccumulation

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

Table A7_4_2-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	No
Vehicle	Yes, DMF (dimethylformamide)
Concentration of vehicle	Approximately 100 µl/L dilution water (= 0.01 vol.%)
Vehicle control performed	Yes, the control aquarium also received an amount of dimethylformamide, which was equivalent to the exposure aquaria.
Other procedures	No

Table A7_4_2-2: Dilution water

Criteria	**Details**
Source	Reconstituted Water
Alkalinity	*Not stated*
Hardness	48 mg $CaCO_3$/L (40 - 60 mg $CaCO_3$/L)
PH	7.0 to 7.2
Oxygen content	*82 % and 104 % saturation*
Conductance	Not stated
Holding water different from dilution water	*No*

Table A7_4_2-3: Test organisms

Criteria	**Details**
Species/strain	*Bluegill Sunfish (*Lepomis macrochirus)
Source	Osage Catfisheries, Inc., Osage Beach, Missouri
Wild caught	*No*
Age/size	Part 1: The mean body wet weight of the fish (lot F 1/05 B) at the beginning of the test was 1.1 ± 0.25 g (Mean ± SD), the mean body length was 4.2 + 0.16 cm (Mean ± SD). Part 2: The mean body wet weight of lot F 2/04 at the beginning of the test was 18.2 ± 5.76 g (Mean + SD), the mean body length was 10.5 ± 0.85 cm (Mean + SD).
Kind of food	*Brutfutter Ecostart 17, BioMar, Denmark.*
Amount of food/ Feeding of animals during test	During the acclimation and Part 1 of the, fish received 2 percent of mean body-weight of a standard fish-feed. Fish in Part 2 of the study received 1 percent of mean body-weight. The amount of feed was re-calculated once per week.
Feeding frequency	*Daily*

Pretreatment	All test fish were held in culture tanks on a 16-hour daylight photoperiod and observed for at least 14 days prior to testing.
Therapeutic or Prophylactic Treatments	Fish received a prophylactic treatment of Oxytetracyclin-Hydrochloride (4g/100L water) following arrival.

Table A7_4_2-4: Test system

Criteria	**Details**
Test type	*Flow-through*
Renewal of test solution	*Not applicable*
Volume of test vessels	*100 litres*
Volume/animal	Part 1: The initial loading was 0.11 - 0.66 g fish/L Part 2: initial loading was 0.91 g fish/L/day.
Number of animals/vessel	*Part 1: 60* *Part 2: 15*
Number of vessels/ concentration	*1*
Test performed in closed vessels due to significant volatility of TS	*Not stated*

Table A7_4_2-5: Test conditions

Criteria	**Details**
Test temperature	*21.5 – 22.7ºC (control aquarium – 21 -23ºC)*
Dissolved oxygen	*82 – 104% saturation*
PH	*7.0 – 7.2*
Adjustment of pH	*Not stated*
Aeration of dilution water	Aerated reconstituted diluent water was used during the study, however the biotransformation test aquaria were specifically aerated during the study.
Intensity of irradiation	*Not stated*
Photoperiod	*Assumed to be 16 hours light daily*

Table A7_4_2-6: Lipid content (whole fish)

Conc. (ng/L)	**Fish sample No.**	**Day 0**		**Day 28**		**Day 42**	
		g/kg fresh weight	**%**	**g/kg fresh weight**	**%**	**g/kg fresh weight**	**%**
Control	1	66.7	6.67	70.2	**7.02**	89.1	8.91
Control	2	63.5	6.35	78.3	7.83	87.2	8.72
Control	3	64.2	6.42	0.5	7.05	91.7	9.17
Control	4	53.3	5.33	51.9	5.19	82.9	8.29
65	1	82.4	8.24	83.3	8.33	91.3	9.13
65	2	74.5	7.45	78.8	7.88	82.5	8.25
65	3	94.1	9.41	65.4	6.54	84.6	8.46
65	4	74.1	7.41	71.4	7.14	97.0	9.70
198	1	52.6	5.26	60.0	6.00	93.4	9.34
198	2	75.8	7.58	58.8	5.88	82.5	8.25
198	3	81.6	8.16	78.7	7.87	97.7	9.77
198	4	40.5	4.05	77.7	7.77	80.5	8.05
Mean		68.6	6.86	70.4	7.04	88.4	8.84
Overall mean		**6.95%**					

Table A7_4_2-7: Mean tissue residues (given in µg/kg fresh weight) at steady state

	Nominal test level of 65 ng [14C]- Transfluthrin/L	**Nominal test level of 198 ng [14C]- Transfluthrin/L**

Edible Parts:	**40.4 µg/kg (based on TRR)**	**114 µg/kg (based on TRR)**
Non Edible Parts:	**233 µg/kg (based on TRR)**	690 µg/kg (based on TRR)
Whole Fish:	**113 µg/kg (based on TRR)**	332 µg/kg (based on TRR)

Table A7_4_2-8: Steady-state, clearance rate constant (K_d) and the time for half clearance for edible parts, viscera and for whole fish (DT_{50})

Parameter (based on TRR)	**Nominal test level of** 65 ng [^{14}C]- Transfluthrin/L			**Nominal test level of** 198 ng [^{14}C]- Transfluthrin/L		
	Edible Parts	Viscera	**Whole Fish**	**Edible Parts**	Viscera	**Whole Fish**
Time to Reach 80% of Steady-State (days)	**10.3**	**8.2**	**8.8**	**9.4**	**6.1**	**7.1**
Time to Reach 95% of Steady-State (days)	**19.2**	**15.3**	**16.5**	**17.6**	**11.4**	**13.2**
t(1/2) for Clearance (days) (DT_{50})	**4.4**	**3.5**	**3.8**	**4.1**	**2.6**	**3.1**
Clearance Rate Constant (K_d) (1/day)	0.156 (± 0.03)	0.196 (± 0.03)	0.182 (± 0.03)	0.171 (± 0.03)	0.263 (± 0.07)	0.227 (± 0.05)

Table A7_4_2-9: BCF values

Calculated BCF values	**Nominal test level of 65 ng [^{14}C]- Transfluthrin/L**	**Nominal test level of** 198 ng [^{14}C]- Transfluthrin/L
$BCF_{TRR\ (whole\ fish,\ wet\ weight)}$	**1704**	**1861**
$BCF_{TRR\ (whole\ fish,\ normalised\ to\ 6\%\ lipid\ content)}$	**1471**	**1607**

4.2.1.2 Study 2 – Bioaccumulation in an appropriate species of fish

Document IIIA/ Section A7.4.3.3.1 BPD Data Set IIIA / Annex Point XIII.2.3	**Bioaccumulation in an appropriate species of fish**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	The proposed uses of transfluthrin in the EU are for insect control in and around homes, e.g. indoor/patio use of electric vaporizers, glowing mosquito coils on terraces and/or anti-moth discs. Therefore, release is on a small scale and entrance to the aquatic environment is very limited resulting in negligible exposure of aquatic organisms. The worst case contamination of surface water results from the use of transfluthrin formulated as 'Raid Portable Electric'. Worst case estimations (relevant Doc IIB, Section 3.3) from the amateur indoor use of transfluthrin in a heated vaporiser (Raid Portable Electric), with subsequent deposition and transfer of residues from room surfaces to wastewater, results in negligible concentrations in surface water (<3.3 x 10^{-8} mg/l). The bioconcentration factor of transfluthrin as examined under flow-through conditions using Bluegill sunfish (*Lepomis macrochirus*) was 1471 – 1607, but significant metabolism into more polar metabolites occurred in the fish tissue. Hence, transfluthrin is not persistent in the fish and will be eliminated if exposure is interrupted (estimated	

Document IIIA/ Section A7.4.3.3.1 BPD Data Set IIIA / Annex Point XIII.2.3	**Bioaccumulation in an appropriate species of fish**	
	clearance time for 50% depuration 3.8 days) (see Doc IIIA, Section 7.4.2). Based upon the very low concentrations in surface water resulting from use of household products a risk for secondary poisoning is not indicated. Following the Technical Guidance Document on Risk Assessment in Support of 98/8/EC, biomagnification in the food chain is also not indicated based upon a BCF < 2000. This is given by a biomagnification factor of 1. Therefore the intrinsic properties of transfluthrin are effectively covered by a) the available data on bioconcentration in fish b) the effects data on aquatic organisms, c) potential predators (bird, mammals), d) transfluthrin is not persistent in fish and e) the risk for secondary poisoning is low. Further data on bioaccumulation in the aquatic system are not therefore deemed necessary.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*02-03-2007*	
Evaluation of applicant's justification	*The available information from the bioconcentration study with Bluegill sunfish is considered sufficient to address the risks of bioaccumulation.*	
Conclusion	*No additional data needed.*	
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		

4.2.2 Bioaccumulation test with other organisms

Document IIIA/ Section A7.4.3.3.2 BPD Data Set IIIA / Annex Point XIII.2.3	**Bioaccumulation in an appropriate invertebrate species**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ] Limited exposure [✓]	Technically not feasible [ ] Scientifically unjustified [✓] Other justification [ ]	
Detailed justification:	The core effects data on fish, daphnia, algae and bacterial populations in sludge result in a $PNEC_{aquatic}$ for transfluthrin of 7.0 x 10^{-7} mg/L based upon the toxicity to rainbow trout (Doc IIA, Section 4.2). Exposure of Bluegill sunfish (*Lepomis macrochirus*) to transfluthrin under flow-through conditions resulted in a BCF of 1471 - 1607 (see Doc IIIA, Section 7.4.2). Significant metabolism of the parent compound in fish tissue was observed and as a consequence, depuration was fast with a clearance half life of ca. 3.8 days. The proposed uses of transfluthrin in the EU are for insect control in and around homes, e.g. indoor/patio use of electric vaporizers, glowing	

Document IIIA/ Section A7.4.3.3.2 BPD Data Set IIIA / Annex Point XIII.2.3	**Bioaccumulation in an appropriate invertebrate species**	
	mosquito coils on terraces and/or anti-moth discs. Therefore, there is no direct release to the aquatic environment. The worst case contamination of surface water was identified as *via* washing of treated room surfaces following use of transfluthrin formulated as 'Raid Portable Electric'. The PEC surface water has been estimated as 3.3 x 10^{-8} mg/L with an associated worst case sediment PEC of 3.6 x 10^{-5} mg/kg (relevant Doc IIB, Section 3.3). From the available data no unacceptable risk to the aquatic environment is shown. The intrinsic properties of transfluthrin are well described by the experimentally derived bioconcentration factor in fish as well as the effects data on algae, fish, daphnia and bacterial populations in sewage sludge. As the outdoor exposure resulting from the use of household devices for insect control is extremely low, the risk to the aquatic environment is minimal. Given that there is also no direct release to surface water additional tests to evaluate the bioaccumulation in aquatic invertebrates are not justified.	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	*02-03-2007*	
Evaluation of applicant's justification	*The available information on bioaccumulation in fish is considered sufficient to address the risks of bioaccumulation.*	
Conclusion	*Further information is not 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		

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

4.3.1.1 Study 1 – acute toxicity to fish - Rainbow trout (Salmo gairdneri)

Document IIIA/ Section A7.4.1.1/01 BPD Data Set IIA / Annex Point VII.7.1	**Acute toxicity to fish Rainbow trout (*Salmo gairdneri*)**	
	Reference	**Official use only**
Data protection	Yes	

Document IIIA/ Section A7.4.1.1/01 BPD Data Set IIA / Annex Point VII.7.1	**Acute toxicity to fish Rainbow trout (*Salmo gairdneri*)**	
Data owner	Bayer CropScience	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	Yes	
	"EEC Directive 79/831, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1. Acute Toxicity for Fish" (published in Amtsblatt der Europaischen Gemeinschaften, Dated 19.09.1984)	
	OECD "Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test".	
GLP	Yes	
Deviations	None	
	Materials and Methods	
Test material	NAK 4455 technical (transfluthrin)	
Lot/Batch number	Mixed pt. 250987	
Specification	As given in section 2	X
Purity	94.5%	X
Composition of Product	Not applicable	
Further relevant properties	Brown solid material	
Method of analysis	Not stated	
Preparation of TS solution for poorly soluble or volatile test substances	See table A7_4_1_1(01)-1	
Reference substance	No	
Method of analysis for reference substance	N/A	
Testing procedure		
Dilution water	See table A7_4_1_1(01)-2	
Test organisms	See table A7_4_1_1(01)-3	
Test system	See table A7_4_1_1(01)-4	
Test conditions	See table A7_4_1_1(01)-5	
Duration of the test	96-hour	
Test parameter	Mortality	
Sampling	Fish were observed twice on the first day of exposure and daily thereafter (at 24, 48, 72 and 96 hours) for mortalities and signs of intoxication.	
	Dissolved oxygen and pH were determined daily, temperature was measured hourly.	
	Water hardness was determined at the beginning and at the end of the test.	
Monitoring of TS concentration	Yes,	
	Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours. Concentrations of 1.58 and 2.81 µg a.i./l were analysed at 0 and 24 hours.	
Statistics	The LC_{50} values with 95%-confidence intervals were calculated by the method of THOMPSON and WEIL (On the Construction of Tables for Moving Average Interpolation, Biometrics, Vol. 8, pp. 51 - 54, 1952) for each 24-hour period if possible. Where the data were inadequate to use statistical methods (0 and 100 % mortality in two adjacent concentrations spaced by a factor of less than 1.8) the LC_{50} is given as	

the geometric mean of the two concentrations and the range between the two respective concentrations is given as 95%-confidence interval.
Results

Limit Test: Not performed

Concentration: N/A

Number/ percentage of animals showing adverse effects: N/A

Nature of adverse effects: N/A

Results test substance

Initial concentrations of test substance: The concentrations tested were: 0.16, 0.28, 0.50, 0.89, 1.58 and 2.81 µg a.i./l (nominal) plus control and solvent control (acetone 0.1 ml/1). X

Actual concentrations of test substance: Analytical results showed that test concentrations were maintained at >80% of the nominal values. Only in the highest concentration the initial measured concentration was 75% of the nominal value but the geometric mean over 24h was above 80% nominal.

Sample timepoints (hours)	Nominal concentrations (µg a.i./l)					
	0.16	0.28	0.50	0.89	1.58	2.81
	Measured concentrations (µg a.i./l)					
0	0.13	0.25	0.52	0.79	1.35	2.12
24	0.14	0.27	0.50	0.84	1.50	2.40
48	0.13	0.29	0.52	0.95	-	-
96	0.13	0.29	0.53	0.90	-	-

The analytical results indicated that the active ingredient was stable in the stock solutions.

Effect data (Mortality): See tables A7_4_1_1(01)-6 and A7_4_1_1(01)-7.

Concentration / response curve: Not reported.

Other effects: Symptoms of intoxication such as swimming on side and/or inverted and staggering were noted in fish at dose levels of 0.89 and 2.81 µg a.i./l.

Results of controls

Number/ percentage of animals showing adverse effects

Nominal Conc. (µg a.i./l)	% mortality				
	4hrs	24hrs	48hrs	72hrs	96hrs
Control	0	0	0	0	0
Solvent control	0	0	0	0	0

Nature of adverse effects: N/A

Test with reference substance: Not performed

Concentrations: N/A

Results: N/A

Applicant's Summary and conclusion

Materials and methods: The study was conducted according to "EEC Directive 79/831, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1. Acute Toxicity for Fish" (published in Amtsblatt der Europaischen

	Gemeinschaften, Dated 19.09.1984) and OECD "Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test". Validity criteria were fulfilled and no deviations were noted. Dates of experimental work: 21/03/1987 to 25/03/1987. Rainbow trout were exposed under flow-through conditions for 96 hours to NAK 4455 technical at nominal concentrations tested of 0.16, 0.28, 0.50, 0.89, 1.58 and 2.81 µg a.i./l. Control and solvent controls (acetone 0.1 ml/1) were also included in the study. Fish were observed twice on the first day of exposure and daily thereafter (at 24, 28, 72 and 96 hours) for mortalities and signs of intoxication. Dissolved oxygen and pH were determined daily, temperature was measured hourly. Water hardness was determined at the beginning and at the end of the test. Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours in the concentrations 0.16, 0.28, 0.50 and 0.89 µg a.i./1. The concentrations 1.58 and 2.81 µg a.i./l were analysed at 0 and 24 hours. Water flow and dosing system were controlled twice daily and water flow was adjusted if necessary.
Results and discussion	Analytical results showed that test concentrations were maintained at >80% of the nominal values. In the highest test concentration, the mean value over 24 h was greater 80% of the nominal concentration with slightly lower values at start of the test. Hence, results refer to nominal values. Mortalities in the control, solvent control, 0.16, 0.28 and 0.50 µg a.i./l concentrations were 0%, respectively. 90% mortality was observed at 0.89 µg a.i./l and 100% mortality was observed at 1.58 and 2.81 µg a.i./l. Symptoms of intoxication such as swimming on side and/or inverted and staggering were noted in fish at dose levels of 0.89 and 2.81 µg a.i./l. Water quality and environmental parameters were within acceptable limits.
LC_0	Not determined, (NOEC 0.5 µg ai/L)
LC_{50}	96 hour value - 0.7 µg a.i./l (95 % confidence intervals 0.62-0.79)
LC_{100}	Not calculated, 100 % observed at 1.58 µg ai/L
Conclusion	The 96-hour LC_{50} of the test substance was calculated to be 0.7 µg a.i./l with a 95%-confidence interval from 0.62 to 0.79 µg a.i./l. The lowest observed effect concentration (LOEC) was 0.89 µg a.i./l. The no-observed effect concentration (NOEC) was 0.50 µg a.i./l. See also validity criteria summarized in table A7_4_1_1(01)-8.
Other Conclusions	The LC_{50} did not decrease significantly with time, the LC50 value after 24 h in flow through was 0.88 µg/L.
Reliability	1
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	26-02-2007
Materials and Methods	Applicant's version is adopted

Results and discussion	Applicant's version is adopted with the following addition: 3.1.2/3.1.3 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (94.5%), but analytical verification shows acceptable recovery.
Conclusion	Applicant's version is adopted The result 96-hours LC_{50} 0.7 µg as/L is used for risk assessment.
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 A7_4_1_1(01)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	Yes
Vehicle	Yes, solvent (acetone) (Stock solution at concentrations of 1.6, 2.8, 5.0, 8.9, 15.8 and 28.1 mg NAK 4455 techn./l. acetone were prepared).
Concentration of vehicle	100 µl acetone/l water in test aquarium
Vehicle control performed	Yes, acetone control
Other procedures	All stock preparations were mixed by a magnetic stirrer during the test.

Table A7_4_1_1(01)-2: Dilution water

Criteria	Details
Source	Reconstituted water aerated to saturation with the following ionic concentrations was used: Ca^{++} : 0.384 mMol/1 Mg^{++} : 0.096 mMol/1 Na^{+} : 0.148 mMol/1 K^{+} : 0.015 mMol/1 Cl^{-} : 0.783 mMol/1 $HC0_3^{-}$: 0.148 mMol/1 SO^{--} : 0.096 mMol/1 The test water was analysed in intervals of ca. 6 months for unwanted contaminants and checked for its suitability by breeding of Daphnia, known to be very sensitive to pollutants.
Alkalinity	Not stated
Hardness	48 (40 - 60) mg $CaC0_3$/l
pH	7.5 to 8.0
Oxygen content	10.6 to 12.7 mg/1
Conductance	Not stated.
Holding water different from dilution water	No

Table A7_4_1_1(01)-3: Test organisms

Criteria	Details
Species/strain	*Rainbow Trout (*Salmo gairdneri*)*
Source	*Forellenzucht LINN, D - 5940 Lennestadt, Germany.*
Wild caught	*No*
Age/size	*Mean body weight at the beginning of the test was 1.2 ± 0.4 (SD) g, mean body length was 4.8 ± 0.4 (SD) cm.*
Kind of food	*Commercial trout diet*
Amount of food	*Not stated*
Feeding frequency	*Not stated*
Pretreatment	*Fish were acclimated to the test water and temperature for at least 14 days*
Feeding of animals during test	*No, fish were not fed 48 hours before and during the study.*

Table A7_4_1_1(01)-4: Test system

Criteria	Details
Test type	*Flow-through*
Renewal of test solution	*Water flow was controlled by a flow meter at a rate of 25 ± 1 1/h. Stock preparations were dosed by HAMILTON Microlab MT dispensers controlled by an EPSON HX-20 computer at a rate of 50 ul/cycle and 72 sec/cycle (ca. 0.1 ml stock preparation/1 water).*
Volume of test vessels	*100-L-aquaria*
Volume/animal	*10 fishes per dose level equals 10 L/fish*
Number of animals/vessel	*10*
Number of vessels/ concentration	*1*
Test performed in closed vessels due to significant volatility of TS	*Not stated*

Table A7_4_1_1(01)-5: Test conditions

Criteria	Details
Test temperature	*Temperature was measured hourly and maintained to 12 ºC throughout test.*
Dissolved oxygen	

Nominal conc. (µg a.i./l)	*Timepoint (hrs)*				
	0	*24*	*48*	*72*	*96*
Control	*12.7*	*12.4*	*12.0*	*11.8*	*10.6*
Solvent control	*12.5*	*12.3*	*11.9*	*12.0*	*10.9*
0.16	*12.3*	*12.0*	*11.6*	*11.6*	*10.8*
0.28	*12.3*	*11.9*	*11.7*	*11.6*	*10.9*
0.50	*12.3*	*11.9*	*11.7*	*11.6*	*11.1*
0.89	*12.3*	*11.9*	*11.7*	*11.8*	*11.2*
1.58	*12.3*	*11.9*	-	-	-
2.81	*12.2*	*11.8*	-	-	-

pH	

Nominal conc. (µg a.i./l)	*Timepoint (hrs)*				
	0	*24*	*48*	*72*	*96*
Control	*8.0*	*7.6*	*7.5*	*7.5*	*7.6*
Solvent control	8.0	7.6	7.5	*7.5*	*7.6*
0.16	8.0	7.6	7.5	*7.6*	*7.6*
0.28	8.0	7.6	7.5	*7.6*	*7.7*
0.50	8.0	7.6	7.5	*7.6*	*7.7*
0.89	8.0	7.6	7.5	*7.6*	*7.7*
1.58	8.0	7.6	-	-	-
2.81	8.0	7.6	-	-	-

Adjustment of pH	*No*
Aeration of dilution water	*Not stated, although oxygen content recorded*
Intensity of irradiation	*Not stated*
Photoperiod	*16 hours light / 8 hours dark*

Table A7_4_1_1(01)-6: Mortality data

Test-Substance Concentration (nominal)[1] [µg a.i./l]	**Mortality and Intoxication**							
	Number (dead/affected)[2]				**Percentage Mortality**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	0/0	0/0	0/0	0/0	0	0	0	0
Solvent control	0/0	0/0	0/0	0/0	0	0	0	0
0.16	0/0	0/0	0/0	0/0	0	0	0	0
0.28	0/0	0/0	0/0	0/0	0	0	0	0
0.50	0/0	0/0	0/0	0/0	0	0	0	0
0.89	5/10	9/10	9/10	9/10	50	90	90	90
1.58	10/10	10/10	10/10	10/10	100	100	100	100
2.81	10/10	10/10	10/10	10/10	100	100	100	100
Temperature [°C]	12							
***pH**	7.6	7.5	7.6	7.7				
***Oxygen [mg/l]**	12.0	11.8	11.7	10.9				

[1] TS concentrations were nominal
[2] Number exposed 10
* mean values

Table A7_4_1_1(01)-7: Effect data

Time	**LC 50 [µg/L]**	**95 % c.l.**
24 h	0.88	0.73-1.07
48 h	0.7	0.62-0.79
96 h	0.7	0.62-0.79
	0.7	0.62-0.79

[1] effect data are based on nominal (n) concentrations

Table A7_4_1_1(01)-8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	**Not fulfilled**
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**yes**	

Criteria for poorly soluble test substances	**yes**	

4.3.1.2 Study 2 – Acute toxicity to fish - Golden Orfe (Leuciscus idus melanotus)

Document IIIA/ Section A7.4.1.1/02 BPD Data Set IIA / Annex Point VII.7.1	**Acute toxicity to fish Golden Orfe (*Leuciscus idus melanotus*)**

	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	*Yes* *"EEC Directive 79/831, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1. Acute Toxicity for Fish" (published in*	

	Amtsblatt der Europäischen Gemeinschaften, Dated 19.09.1984) *OECD "Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test".*
GLP	*Yes*
Deviations	Yes In the lowest test concentration (0.50 µg a.i/l) only 58 to 74 % of the nominal concentration was found following analysis. Although the concentration of the test substance is <80% of nominal it is considered that there is no influence on the validity of the study, because in the next highest concentration (0.89 µg a.i./l) no effects were observed and mean measured concentrations were >80%.
	Materials and Methods
Test material	*NAK 4455 technical (transfluthrin)*
Lot/Batch number	*Mixed pt. 250987*
Specification	*Identified by Batch Number* As given in section 2
Purity	*94.5%*
Composition of Product	*Not applicable*
Further relevant properties	*Brown solid material*
Method of analysis	*Test concentrations were determined by gas chromatography.*
Preparation of TS solution for poorly soluble or volatile test substances	*See table A7_4_1_1(02)-1*
Reference substance	*No*
Method of analysis for reference substance	*N/A*
Testing procedure	
Dilution water	*See table A7_4_1_1(02)-2*
Test organisms	*See table A7_4_1_1(02)-3*
Test system	*See table A7_4_1_1(02)-4*
Test conditions	*See table A7_4_1_1(02)-5*
Duration of the test	*96-hour*
Test parameter	*Mortality*
Sampling	*Fish were observed twice on the first day of exposure and daily thereafter (at 24, 48, 72 and 96 hours) for mortalities and signs of intoxication.* *Dissolved oxygen and pH were determined daily, temperature was measured hourly.* *Water hardness was determined at the beginning and at the end of the test.*
Monitoring of TS concentration	*Yes,* *Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours at concentrations of 0.50, 0.89 and 2.81 µg a.i./l. Concentrations of 1.58 and 5.00 µg a.i./l were only analysed at 0 and 24 hours.*
Statistics	*The LC_{50} values with 95%-confidence intervals were calculated by the method of THOMPSON and WEIL (On the Construction of Tables for Moving Average Interpolation, Biometrics, Vol. 8, pp. 51 - 54, 1952) for each 24-hour period if possible. Where the data were inadequate to use statistical methods (0 and 100 % mortality in two adjacent concentrations spaced by a factor of less than 1.8) the LC_{50} is given as the geometric mean of the two concentrations and the range between the two respective concentrations is given as 95 %-confidence interval.*

Results

Limit Test: *Not performed*

Concentration: *N/A*

Number/ percentage of animals showing adverse effects: *N/A*

Nature of adverse effects: *N/A*

Results test substance

Initial concentrations of test substance: *The concentrations tested were: 0.50, 0.89, 1.58, 2.81 and 5.00 µg a.i./l (nominal) plus control and solvent control (acetone 0.1 ml/1).*

Actual concentrations of test substance: *Analytical results showed that test concentrations of 0.89, 1.58 and 2.81 µg a.i./l were maintained at a mean level of >80% of the nominal values. In the highest concentration (5.00 µg a.i./l) the initial measured concentration was slightly below 80% of the nominal value. In the lowest concentration only 58-74% of the nominal concentration was recovered by analysis. It is considered that this had no influence on the study validity as at the next highest concentration 0.89 µg a.i./l no effects were observed.* X

Sample timepoints (hours)	*Nominal concentrations (µg a.i./l)*				
	0.50	*0.89*	*1.58*	*2.81*	*5.00*
	Measured concentrations (µg a.i./l)				
0	*0.34*	*0.73*	*1.40*	*2.38*	*3.15*
24	*0.35*	*0.65*	*1.54*	*2.48*	*4.26*
48	*0.29*	*0.75*	*-*	*2.55*	*-*
96	*0.37*	*0.76*	*-*	*2.50*	*-*

Effect data (Mortality): *See tables A7_4_1_1(02)-6 and A7_4_1_1(02)-7.*

Concentration / response curve: *Not reported.*

Other effects: *Symptoms of intoxication such as swimming on side and/or inverted, red marks on the skin and swimming behaviour slightly irregular (only at 1.58 µg a.i/l) were noted in fish at dose levels of 1.58, 2.81 and 5.00 µg a.i./l.*

Results of controls

Number/ percentage of animals showing adverse effects

Test system	*% mortality*				
	4hrs	*24hrs*	*48hrs*	*72hrs*	*96hrs*
Control	*0*	*0*	*0*	*0*	*0*
Solvent control	*0*	*0*	*0*	*0*	*0*

Nature of adverse effects: *N/A*

Test with reference substance: *Not performed*

Concentrations: *N/A*

Results: *N/A*

Applicant's Summary and conclusion

Materials and methods: *The study was conducted according to "EEC Directive 79/831, Annex V, Methods for Determination of Ecotoxicity, Method 5.1.1. Acute Toxicity for Fish" (published in Amtsblatt der Europaischen Gemeinschaften,*

	Dated 19.09.1984) and OECD "Guideline for Testing of Chemicals, No. 203, Fish, Acute Toxicity Test". Validity criteria were fulfilled and minor deviations were noted. Dates of experimental work: 21/03/1988 to 25/03/1988. *Golden orfe were exposed under flow-through conditions for 96 hours to NAK 4455 technical at nominal concentrations tested of 0.50, 0.89, 1.58, 2.81and 5.00 µg a.i./l. Control and solvent controls (acetone 0.1 ml/1) were also included in the study. Analytical measurements of the active ingredient were done at 0, 24, 48 and 96 hours in the concentrations 0.50, 0.89 and 2.81µg/1. The concentrations 1.58 and 5.00 µg/1 were only analysed at 0 and 24 hours. Analytical results showed that test concentrations of 0.89, 1.58 and 2.81 µg a.i./l were maintained at a mean level of >80% of the nominal values. In the highest concentration (5.00 µg a.i./l) the initial measured concentration was slightly below 80 % of the nominal value. In the lowest concentration only 58-74% of the nominal concentration was recovered by analysis. It is considered that this had no influence on the study validity as at the next highest concentration 0.89 µg a.i./l no effects were observed. Hence, results of the study are reported as nominal concentrations.* *Fish were observed twice on the first day of exposure and daily thereafter (at 24, 28, 72 and 96 hours) for mortalities and signs of intoxication. Dissolved oxygen and pH were determined daily, temperature was measured hourly. Water hardness was determined at the beginning and at the end of the test.* *Water flow and dosing system were controlled twice daily and water flow was adjusted if necessary.*
Results and discussion	*Mortalities in the control, solvent control, 0.50 and 0.89 µg a.i./l concentrations were 0%, respectively. 90% mortality was observed at 2.81 µg a.i./l and 100% mortality was observed at 1.58 and 5.00 µg a.i./l. Symptoms of intoxication such as swimming on side and/or inverted, red marks on the skin and swimming behaviour slightly irregular (only at 1.58 µg a.i./l) were noted in fish at dose levels of 1.58, 2.81 and 5.00 µg a.i./l.* *Water quality and environmental parameters were within acceptable limits.*
LC_0	*Not determined.*
LC_{50}	*96 hour value -1.25 µg a.i./l (95 % confidence intervals 1.1-1.4)*
LC_{100}	*Not determined.*
Conclusion	*The 96-hour LC_{50} based upon nominal concentrations of the test substance was calculated to be 1.25 µg a.i./l with a 95 %-confidence interval from 1.1 to 1.4 µg a.i./l. The lowest observed effect concentration (LOEC) was 1.58 µg a.i./l. The no-observed effect concentration (NOEC) was 0.89 µg a.i./l.* *See also validity criteria summarized in table A7_4_1_1(02)-8.*
Other Conclusions	*Mortality did not increase from 24h to 96 hours, the LC50 at 24 h equals that of 96h*
Reliability	*1*
Deficiencies	*Minor. The test organism used is not a standard fish species suggested acc. to OECD 203 but the presented result gives a valid endpoint for an additional fish species.*

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the

	comments and views submitted
	Evaluation by Rapporteur Member State
Date	*26-02-2007*
Materials and Methods	*Applicant's version is adopted with the following addition:* *3.1.2/3.1.3 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (94.5%), but analytical verification shows acceptable recovery.*
Results and discussion	*Applicant's version is adopted with the following addition:* *4.2.2: recovery at the lowest and highest concentration is < 80%, but since at the level of the LC50 recovery was acceptable, expression on the basis of nominal concentrations is accepted.*
Conclusion	*Applicant's version is adopted* *The result 96-hours LC_{50} 1.25 µg as/L is used for risk assessment.*
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 A7_4_1_1(02) -1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	*Yes*
Vehicle	*Yes, solvent (acetone)* *(Stock solution at concentrations of 5.0, 8.9, 15.8, 28.1 and 50 mg NAK 4455 techn./l. acetone were prepared).*
Concentration of vehicle	*see above, dose to 100 µl acetone/l aquarium water*
Vehicle control performed	*Yes, acetone control*
Other procedures	*All stock preparations were mixed by a magnetic stirrer during the test.*

Table A7_4_1_1(02) -2: Dilution water

Criteria	Details
Source	*Reconstituted water aerated to saturation with the following ionic concentrations was used:* *Ca^{++}: 0.384 mMol/1* *Mg^{++} : 0.096 mMol/1* *Na^{+} : 0.148 mMol/1* *K^{+} : 0.015 mMol/1* *Cl^{-} : 0.783 mMol/1* *$HC0_3^{-}$: 0.148 mMol/1* *SO_4^{--} : 0.096 mMol/1* *The test water was analysed in intervals of ca. 6 months for unwanted contaminants and checked for*

	its suitability by breeding Daphnia as a very sensitive species for pollutants.
Alkalinity	*Not stated*
Hardness	*48 -50 mg $CaC0_3$/l*
pH	7.4 to 8.1
Oxygen content	*9.1 to 11.5 mg/1*
Conductance	Not stated
Holding water different from dilution water	*No*

Table A7_4_1_1(02) -3: Test organisms

Criteria	**Details**
Species/strain	*Golden orfe (*Leuciscus idus melanotus*)*
Source	*Fischzucht EGGERS, D - 2354 Hohenwestedt, Germany.*
Wild caught	*No*
Age/size	*Mean body weight at the beginning of the test was 3.5 ± 0.5 (SD) g, mean body length was 7.2 ± 0.3 (SD) cm.*
Kind of food	*Commercial trout diet*
Amount of food	*Not stated*
Feeding frequency	*Not stated*
Pretreatment	*Fish were acclimated to the test water and temperature for at least 14 days*
Feeding of animals during test	*No, fish were not fed 48 hours before and during the study.*

Table A7_4_1_1(02) -4: Test system

Criteria	**Details**
Test type	*Flow-through*
Renewal of test solution	*Water flow was controlled by a flow meter at a rate of 25 ± 1 1/h. Stock preparations were dosed by HAMILTON Microlab MT dispensers controlled by an EPSON HX-20 computer at a rate of 50 µl/cycle and 72 sec/cycle (ca. 0.1 ml stock preparation/1 water).*
Volume of test vessels	*100-L-aquaria*
Volume/animal	*10 fishes per 100 L aquarium refers to 10L/fish*
Number of animals/vessel	*10*
Number of vessels/ concentration	*1*
Test performed in closed vessels due to significant volatility of TS	*Not stated*

Table A7_4_1_1(02) -5: Test conditions

Criteria	**Details**
Test temperature	*19 - 20 ºC*
Dissolved oxygen	

Nominal conc. (µg a.i./l)	*Timepoint (hrs)*				
	0	*24*	*48*	*72*	*96*
Control	*11.0*	*10.7*	*10.3*	*10.3*	*9.2*
Solvent control	*11.0*	*10.6*	*10.2*	*10.2*	*9.1*
0.50	*11.1*	*10.6*	*10.3*	*10.2*	*9.1*
0.89	*11.1*	*10.6*	*10.3*	*10.2*	*9.2*
1.58	*11.5*	*10.8*	-	-	-

	2.81	*11.4*	*11.0*	*10.6*	*10.6*	*9.9*
	5.00	*11.3*	*11.0*	-	-	-

pH	*Nominal conc. (µg a.i./l)*	*Timepoint (hrs)*				
		0	*24*	*48*	*72*	*96*
	Control	*8.1*	*7.4*	*7.5*	*7.6*	7.6
	Solvent control	8.1	7.4	7.5	7.6	7.6
	0.50	8.1	7.4	7.5	7.6	7.6
	0.89	8.1	7.4	7.5	7.6	7.6
	1.58	8.1	7.4	-	-	-
	2.81	8.1	7.4	7.5	7.6	7.6
	5.00	8.1	7.4	-	-	-
Adjustment of pH	*No*					
Aeration of dilution water	*Not stated, flow rate was 25 + 1 L/h*					
Intensity of irradiation	*Not stated*					
Photoperiod	*16 hours light / 8 hours dark*					

Table A7_4_1_1(02) -6: Mortality data

Test-Substance Concentration (nominal)[1] [µg a.i./l]	**Mortality and Symptoms of Intoxication**							
	Number (dead/affected)[2]				**Percentage mortality**			
	24 h	**48 h**	**72 h**	**96 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	0/0	0/0	0/0	0/0	0	0	0	0
Solvent control	0/0	0/0	0/0	0/0	0	0	0	0
0.50	0/0	0/0	0/0	0/0	0	0	0	0
0.89	0/0	0/0	0/0	0/0	0	0	0	0
1.58	10/10	10/10	10/10	10/10	100	100	100	100
2.81	9/10	9/10	9/10	9/10	90	90	90	90
5.00	10/10	10/10	10/10	10/10	100	100	100	100
Temperature [°C]	19-20							
***pH**	7.4	7.5	7.6	7.6				
***Oxygen [mg/l]**	10.8	10.3	10.3	9.3				

[1] TS concentrations were nominal

[2] Number exposed 10, effects were swimming on side and/or inverted and, red marks on the skin at 24 h in the 2.81 µg/L dose

* mean values

Table A7_4_1_1(02) -7: Effect data

Time	**LC_{50} [µg/l][1]**	**95 % c.l.**
24h	1.25	1.1-1.4
48h	1.25	1.1-1.4
96h	1.25	1.1-1.4

[1] effect data are based on nominal (n) concentrations

Table A7_4_1_1(02) -8: Validity criteria for acute fish test according to OECD Guideline 203

	fulfilled	**Not fulfilled**
Mortality of control animals <10%	**yes**	
Concentration of dissolved oxygen in all test vessels > 60% saturation	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**in part**	

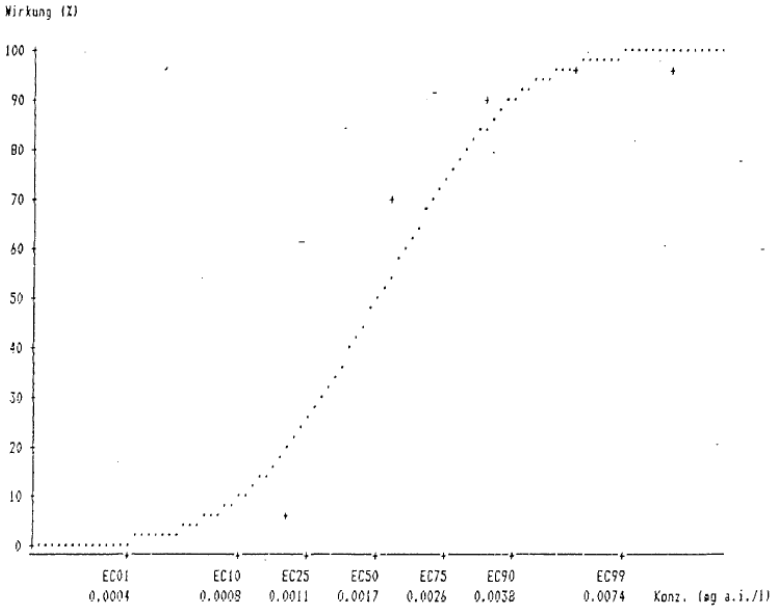
Criteria for poorly soluble test substances	**yes**	

4.3.2 Short-term toxicity to aquatic invertebrates

4.3.2.1 Study 1 – acute toxicity to invertebrates – Daphnia magna

Document IIIA/ Section A7.4.1.2/01 BPD Data Set IIA / Annex Point VII.7.2	**Acute toxicity to invertebrates Daphnia magna**

	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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	
	Guidelines and Quality Assurance	
Guideline study	*Yes*	
	OECD Guideline No. 202 'Guideline for Testing of Chemicals', 'Daphnia sp.*, Acute Immobilisation Test and Reproduction Test, Part I, Adopted 4 April 1984'.*	
GLP	*Yes*	
Deviations	None	
	Materials and Methods	
Test material	*NAK 4455 (transfluthrin technical)*	
Lot/Batch number	*130187*	X
Specification	As given in section 2	X
Purity	*95.0%*	
Composition of Product	*N/A*	
Further relevant properties	*Appearance: brown liquid*	
Method of analysis	*Not stated*	
Preparation of TS solution for poorly soluble or volatile test substances	*See table A7_4_1_2(01)-1*	
Reference substance	*Yes, potassium dichromate (test conducted in a separate test on 15/01/1987)*	
Method of analysis for reference substance	*Not stated*	
Testing procedure		
Dilution water	*See table A7_4_1_2(01)-2*	
Test organisms	*See table A7_4_1_2(01)-3*	
Test system	*See table A7_4_1_2(01)-4*	
Test conditions	*See table A7_4_1_2(01)-5*	
Duration of the test	*48 hours*	
Test parameter	*Immobility*	
Sampling	*Visual observations of immobility were made at 24 and 48 hour (unconfirmed immobilisation was verified by use of a microscope).*	
Monitoring of TS concentration	*No*	
Statistics	*Probit Analysis -"Maximum Likelihood "Method.*	
	Results	
Limit Test	*Not performed*	
Concentration	*N/A*	
Number/ percentage of animals showing adverse	*N/A*	

effects	
Nature of adverse effects	*N/A*
Results test substance	
Initial concentrations of test substance	*0.010, 0.0056, 0.0032, 0.0018 and 0.0010 mg a.i./l (control and solvent controls were also included).*
Actual concentrations of test substance	*Test reported in nominal concentrations.*
Effect data (Immobilisation)	*See tables A7_4_1_2(01)-6 and A7_4_1_2(01)-7*
Concentration / response curve	
Other effects	*Sub-lethal effects reported were rapid trembling of antennae, stagnation at water surface, animals on bottom of vessel and/or almost immobile as given in Table A7_4_1_2(01)-6.*
Results of controls	*0% mortality was observed in the control (dilution water only) and 3% mortality was observed in the solvent control (0.1 ml/l acetone) after 24h.*
Test with reference substance	*Performed in a separate test (GLP, E 320 0017-3)*
Concentrations	*0.75, 1.00, 1.33, 1.73, 2.37 and 3.16 mg/1*
Results	*24-hour EC_{50} value was 1.72 mg/L (95% confidence intervals 1.54 – 1.91 mg/l).*
	Applicant's Summary and conclusion
Materials and methods	*The study was conducted in accordance with OECD Guideline No. 202 'Guideline for Testing of Chemicals',* 'Daphnia sp.*, Acute Immobilisation Test and Reproduction Test, Part I, Adopted 4 April 1984'.* *Juvenile* Daphnia magna *(6 -24 hours old) were exposed for 48 hours under static test conditions to NAK 4455 (transfluthrin technical) at nominal concentrations of 0.0010, 0.0018, 0.0032, 0.0056 and 0.010 mg a.i./l. Control and solvent controls were also included in the study.* *Daphnids were observed for immobilisation and sublethal effects at 24 and 48 hours. Dissolved oxygen, temperature and pH were measured at the start and end of the study.*
Results and discussion	*Mortalities in the control and solvent control were 0 and 3%, respectively. After 24 h, 3%, 7% and 10 % of the daphnids were immobile at the test concentration of 0.0018, 0.0032, 0.0056 mg a.i./L. No effects were observed at 0.01 mg a.i./L at this time point. 7, 70 and*

	90% immobilisation was observed at the 0.0010, 0.00018 and 0.0032 mg a.i/l test concentrations after 48 h; 97% mortality was observed at the 0.0056 and 0.010 mg a.i./l test concentrations respectively. *Water quality and environmental parameters were within acceptable limits.*
EC_{50}	*The EC_{50} at 24h was 0.039 mg a.i./L (95% confidence intervals of 0.0003-0.003 mg a.i./l), the 48 hour value was 0.0017 mg a.i./l (95% confidence intervals of 0.0003-0.003 mg a.i./l).*
Conclusion	The 48-hour EC_{50} of NAK 4455 to Daphnia magna was calculated to be 0.0017 mg a.i./l with 95%-confidence intervals of 0.0003 to 0.003 mg a.i./l. The 'no-observed-effect-concentration' (NOEC) (48 hours) was 0.0001 mg a.i./l. The 'lowest lethal concentration' (LLC) was 0.001 mg a.i./l. See also validity criteria summarized in table A7_4_1_2(01)-8.
Reliability	*2*
Deficiencies	No analysis of the actual test concentration.

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*26-02-2007*
Materials and Methods	*Applicant's version is adopted with the following addition:* *3.1.2/3.1.3 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (95%), but this was most likely accounted for when preparing the test solutions (EC_{50} reported as 0.0017 mg a.i./L).*
Results and discussion	*Applicant's version is adopted*
Conclusion	*Applicant's version is adopted* *The result 48-hours EC_{50} 1.7 µg as/L is used for risk assessment.*
Reliability	*2* *no verification of test concentrations*
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 A7_4_1_2(01)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	*Yes, magnetic stirrer (approx. 60 minutes)*
Vehicle	*Yes, solvent (acetone)*
Concentration of vehicle	*0.1 ml/l*
Vehicle control performed	*Yes (acetone control)*
Other procedures	*None*

Table A7_4_1_2(01)-2: Dilution water

Criteria	Details
Source	*Deionised water*
Alkalinity	*Not stated*
Hardness	*Not stated*
pH	8.00
Ca / Mg ratio	*$CaCl_2$ x 2 H_20 p.a. (0.08 mol/1)/ $MgSO_4$ x 7 H_20 p.a. (0.02 mol/1)*
Na / K ratio	*$NaHCO_3$ p.a. (0.03 mol/1) / KC1 p.a. (0.003 mol/l)*
Oxygen content	*95.9%*
Conductance	Not stated
Holding water different from dilution water	*No*

Table A7_4_1_2(01)-3: Test organisms

Criteria	Details
Strain	Daphnia maqna
Source	*Bundesgesundheitsamt, Berlin*
Age	*6-24 hours old*
Breeding method	*Not stated (strain bred in laboratory for a long period of time).*
Kind of food	*Algae (*Scenedesmus subspicatus*) and fish flake (TetraMin®).*
Amount of food	*Not stated*
Feeding frequency	*Not stated*
Pretreatment	Daphnia *were held in the laboratory under standard conditions i.e. 20 ± °C, 16:8 light/dark cycle, which are identical to the test conditions.*
Feeding of animals during test	*No*

Table A7_4_1_2(01)-4: Test system

Criteria	Details
Renewal of test solution	*Static test*
Volume of test vessels	*100 mL (containing 50 mL of test medium)*
Volume/animal	*5ml/1 organism*
Number of animals/vessel	*10*
Number of vessels/ concentration	*3*
Test performed in closed vessels due to significant volatility of TS	*Yes, test vessels were covered with watch glass lids (60 mm in diameter).*

Table A7_4_1_2(01)-5: Test conditions

Criteria	Details
Test temperature	*19.6 °C*
Dissolved oxygen	*98.8 – 99.9%*
pH	*7.93 – 8.00*
Adjustment of pH	*No*
Aeration of dilution water	*No*
Quality/Intensity of irradiation	*Not stated*
Photoperiod	*16:8 hours light:dark cycle*

Table A7_4_1_2(01)-6: Immobilisation and sublethal effects data

Test-Substance Concentration (nominal) [1] [mg a.i./l]							
	Immobile *Daphnia**						
	Number		Percentage		Oxygen [%]	pH	Temperature [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h

Control	0	0	0	0	99.9	8.00	19.6
Solvent control	0	1	0	3	99.9	7.97	
0.0010	0[3,4]	2[3,4]	0	7	98.9	7.94	
0.0018	1[3,4]	21[3,4]	3	70	-	-	
0.0032	2[2,3,4,5]	27[3,4]	7	90	-	-	
0.0056	3[2,3,4,5]	29[3,4]	10	97	-	-	
0.010	0[2,3,4,5]	29[3,4]	0	97	99.5	7.93	

[1] TS concentrations are based on nominal
[2] stagnation of animals on water surface
[3] animals on bottom of test vessels
[4] almost immobile
[5] rapid trembling of antenna
*(30 organisms exposed in total per concentration)

Table A7_4_1_2(01)-7: Effect data

	EC_{50}[1]	95 % c.l.
24 h [mg a.i./l]	0.039	0.017-0.17
48 h [mg a.i./l]	0.0017	0.0003-0.003

[1] effect data are based on nominal (n) concentrations

Table A7_4_1_2(01)-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	Yes	
Control animals not staying at the surface	Yes	
Concentration of dissolved oxygen in all test vessels >3 mg/l	Yes	
Concentration of test substance ≥80% of initial concentration during test	N.D.	

Criteria for poorly soluble test substances	Yes	

N.D. – Not determined

4.3.2.2 Study 2 - Acute toxicity to invertebrates – Daphnia magna

Document IIIA/ Section A7.4.1.2/02 BPD Data Set IIA / Annex Point VII.7.2	**Acute toxicity to invertebrates Daphnia magna**

	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	*Yes* *EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 'Acute toxicity for* Daphnia*' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 202 'Daphnia sp., Acute Immobilisation Test and Reproduction Test, Part I.*	
GLP	*Yes*	

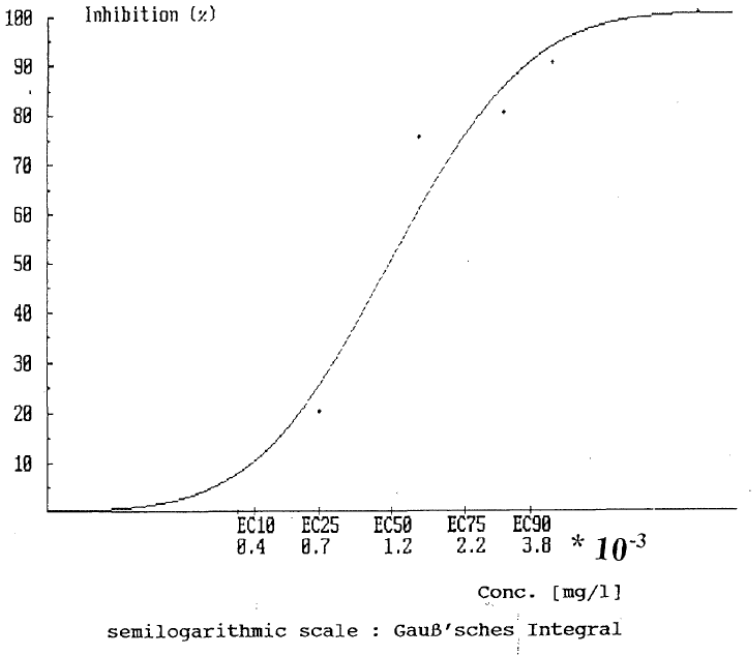
Document IIIA/ Section A7.4.1.2/02 BPD Data Set IIA / Annex Point VII.7.2	**Acute toxicity to invertebrates Daphnia magna**	
Deviations	None	
	Materials and Methods	
Test material	*NAK 4455 (transfluthrin technical)*	
Lot/Batch number	*816779502*	X
Specification	As given in section 2	X
Purity	*95.7%*	
Composition of Product	*N/A*	
Further relevant properties	*Molecular weight: 371 g/mol* *Water solubility: 5.7 x 10^{-5} g/l* *Vapour pressure: 4.0 x 10^{-6} hPa (20°C)10^{-5}*	
Method of analysis	*Stability of test concentrations was verified by chemical analysis using Gas Chromatography (GC).*	
Preparation of TS solution for poorly soluble or volatile test substances	*See table A7_4_1_2(02)-1*	
Reference substance	*No*	
Method of analysis for reference substance	*N/A*	
Testing procedure		
Dilution water	*See table A7_4_1_2(02)-2*	
Test organisms	*See table A7_4_1_2(02)-3*	
Test system	*See table A7_4_1_2(02)-4*	
Test conditions	*See table A7_4_1_2(02)-5*	
Duration of the test	*48 hours*	
Test parameter	*Immobility*	
Sampling	*Visual observations of immobility were made at 24 and 48 hours.*	
Monitoring of TS concentration	*Yes, samples from test concentrations above 0.0008 mg/L were taken at 0 and 48 hours and analysed using Gas Chromatography (GC). GC values were calculated (corresponding to the analytical recovery rate of the highest test concentration) at 0.0002, 0.0004 and 0.0008 mg a.i./1 as the concentrations were below the quantitation limit of the GC method (0.001 mg a.i./1).*	X
Statistics	*Probit Analysis*	
	Results	
Limit Test	*Not performed*	
Concentration	*N/A*	
Number/ percentage of animals showing adverse effects	*N/A*	
Nature of adverse effects	*N/A*	
Results test substance		
Initial concentrations of test substance	*Control, 0.0002, 0.0004, 0.0008, 0.002, 0.004, 0.008, 0.02 and 0.04 mg a.i./l .*	
Actual concentrations of test substance	Measured concentrations ranged from 75 - 123% of nominal values at 0 hours and from 38 - 50% of nominal values at 48 hours, respectively. At test concentrations of 0.0002, 0.0004 and 0.0008 mg a.i./1 GC values were calculated (corresponding to the analytical recovery rate of the highest test concentration) as the concentrations were below the quantitation limit of the GC method (0.001 mg a.i./1).	X

Test concentration (mg a.i/l)	*0 hours*	*48 hours*
Control	*<0.001*	*<0.001*
0.0002	*0.000245*	*0.00008*

0.0004	*0.00049*	*0.00016*
0.0008	*0.00098*	*0.00032*
0.002	*0.002*	*0.001*
0.004	*0.004*	*0.002*
0.008	*0.006*	*0.003*
0.02	*0.022*	*0.008*
0.04	*0.049*	*0.016*

Effect data (Immobilisation) *See tables A7_4_1_2(02)-6 and A7_4_1_2(02)-7*

Concentration / response curve *Concentration-immobilisation curve for* Daphnia magna *exposed for 48h to NAK 4455 (Bayothrin):*
Correlation coefficient: 0.665



Other effects *None*

Results of controls *0% mortality was observed in the controls (dilution water only).*

Test with reference substance *Not performed*

Concentrations *N/A*

Results *N/A*

Applicant's Summary and conclusion

Materials and methods *The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 2 'Acute toxicity for* Daphnia*' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 202* 'Daphnia sp.*, Acute Immobilisation Test and Reproduction Test, Part I. Dates of experimental work: 27/06/2001 to 29/06/2001.*
Juvenile Daphnia magna *(<24 hours old) were exposed for 48 hours under static test conditions to NAK 4455 (transfluthrin technical) at nominal concentrations of 0.0002, 0.0004, 0.0008, 0.002, 0.004, 0.008, 0.02 and 0.04 mg a.i./l. An untreated control was also included in the study.*
Daphnids were observed after 24 and 48 hours for alteration of mobility

	and loss of locomotory actions. Dissolved oxygen, temperature and pH were measured at the end of the study. Water hardness was determined at the beginning of the test. Analytical samples were taken from the controls and the 0.002, 0.004, 0.008, 0.02 and 0.04 mg a.i./l test concentrations at 0 and 48 hours and analysed using GC. The test concentrations 0.0002, 0.0004 and 0.0008 mg a.i./1 were not analytically determined as they were below the quantitation limit of the GC analysis method (0.001 mg/1). However at these test concentrations GC values were calculated (corresponding to the analytical recovery rate of the highest test concentration).	X
Results and discussion	*Mean measured concentrations ranged from 75 - 123% of nominal values at 0 hours and from 38 - 50% of nominal values at 48 hours, respectively. The results are expressed as nominal values for the 24h effect and as mean measured concentrations for the 48h EC50 value.* *No immobilisation was recorded in the control, 0.0002 or 0.0004 mg a.i./l test concentrations. 20, 75, 80, 90 and 100% mortality was observed at the 0.0008, 0.002, 0.004, 0.008, 0.02 and 0.004 mg a.i/l test concentrations, respectively. The EC50 at 24 h was > 0.013 mg/L, the EC50 at 48h was 0.0012 mg/L (probit analysis).* *Water quality and environmental parameters were within acceptable limits.*	X
EC_0	*(based on mean measured) 48 hour value - 0.00033 mg a.i./l*	
EC_{50}	*(based on mean measured) 48 hour value - 0.0012 mg a.i./l (95% confidence intervals of 0.0008-0.0016).*	
EC_{100}	*(based on mean measured) 48 hour value - 0.019 mg a.i./l*	
Conclusion	*The 48-hour EC_{50} of NAK 4455 to* Daphnia magna *was calculated to be 0.0012 mg a.i./l with 95%-confidence intervals of 0.0008 to 0.0016 mg a.i./l. See also validity criteria summarized in table A7_4_1_2(02)-8.*	
Reliability	*1*	
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	*26-02-2007*
Materials and Methods	*Applicant's version adequately reflects the report. The following comments can be made:* *3.1.2/3.1.3 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (94.5%), but concentrations were measured.* *3.4.8 For the analytical measurements, only the resulting figures are given. Method and results are not described in detail (i.e. it is not clear whether samples were analysed in duplicate, recovery is given as 98 %, but spiking level and individual data are not given).*

Results and discussion	*Applicant's version adequately reflects the report. The following comments can be made:* *4.2.2 Actual concentrations at t = 0 were 100, 100, 75, 110 and 123 % of nominal at 2 to 40 µg as/L (average 102 %). At 48 hours, actual concentrations were 50, 50, 37.5, 40 and 40 % of nominal (average 43.5 %). The 24- and 48-hours concentrations at 0.2, 0.4 and 0.8 µg/L nominal were calculated by correction with the recovery of the highest exposure level, i.e. 123 and 40 %, respectively. Since the range of recoveries is relatively small, it is more appropriate to use the average recoveries of 102 and 43.5 %.* *5.2 The 48-hours EC_{50} on the basis of nominal concentrations is 1.6 µg/L (trimmed Spearman-Kärber method). Using the average recovery at 24 and 48 hours, the estimated concentration over the duration of the test is 1.2 µg/L. This value is the same as derived by the authors, although slightly differently calculated.*
Conclusion	*The result 48-hours EC_{50} 1.2 µg/L (estimated concentration during the test) is used for risk assessment*
Reliability	*2* *Actual concentrations at lower exposure levels estimated instead of measured.*
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 A7_4_1_2(02)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	*Yes, A stock solution was prepared to give the desired series of test concentrations. To achieve this 1.5 mg of the test substance were added to 3 litres of dilution water and treated for 60 seconds with an ultra turrax and afterwards stirred for 24 h on a magnetic stirrer. Finally undissolved particles of the test substance were removed by filtration.*
Vehicle	*No (dilution water only)*
Concentration of vehicle	*n/a*
Vehicle control performed	*n/a*
Other procedures	*None*

Table A7_4_1_2(02)-2: Dilution water

Criteria	Details
Source	*Reconstituted water ('M4 medium', originally described in Water Research 24 (9): 1157-1167), prepared according to the recommendations of Bundesgesundheitsamt Berlin.*

Alkalinity	*Not stated*
Hardness	*273.1 mg/l $CaCO_3$*
pH	8.0
Ca / Mg ratio	*Not stated*
Na / K ratio	*Not stated*
Oxygen content	*8.5 mg/L (93.1% saturation)*
Conductance	Not stated
Holding water different from dilution water	*No*

Table A7_4_1_2(02)-3: Test organisms

Criteria	**Details**
Strain	Daphnia magna *STRAUS*
Source	*Bundesgesundheitsamt, Berlin*
Age	*Neonates <24 hours old*
Breeding method	*A population of parthenogenetic females of synchronized age structure has been maintained for more than 15 years in the test facility under constant temperature conditions (20 ± 1 °C) at a 16:8 hour light-dark photoperiod (illumination: <1000 lux). The culture water ('M4 medium') is partly renewed once a week. The mortalities of parent* Daphnia *during the culture period are recorded daily. The neonates are separated from their parent* Daphnia *by filtration prior to an acute test.*
Kind of food	Daphnia *were exclusively fed with unicellular green algae (*Scenedesmus subspicatus *CHODAT).*
Amount of food	'ad libitum'
Feeding frequency	*Not stated*
Pretreatment	Daphnia *were held in the laboratory under standard conditions i.e. 20 ± 1 °C, 16:8 light/dark cycle, which are identical to the test conditions.*
Feeding of animals during test	*No*

Table A7_4_1_2(02)-4: Test system

Criteria	**Details**
Renewal of test solution	*Static test*
Volume of test vessels	*50 mL (containing 20 mL of test medium)*
Volume/animal	*2ml/1 organism*
Number of animals/vessel	*10*
Number of vessels/ concentration	*2*
Test performed in closed vessels due to significant volatility of TS	*Not stated*

Table A7_4_1_2(02)-5: Test conditions

Criteria	**Details**
Test temperature	*Range 19.9 -20 °C*
Dissolved oxygen	*8.4 – 8.5 mg/l (approx. 90 -93% saturation)*
pH	*7.9 – 8.0*
Adjustment of pH	*No*
Aeration of dilution water	*No*
Quality/Intensity of irradiation	*< 1000 lux*
Photoperiod	*16:8 hours light:dark cycle*

Table A7_4_1_2(02)-6: Immobilisation data

Test-Substance Concentration (nominal) [1] [mg a.i./l]	Immobile *Daphnia**						
	Number		Percentage		Oxygen [mg/l]	pH	Temperature [°C]
	24 h	48 h	24 h	48 h	48 h	48 h	48 h
Control	0	0	0	0	8.5	8.0	20.0
0.0002	0	0	0	0	8.4	8.0	20.0
0.0004	0	0	0	0	8.5	8.0	19.9
0.0008	0	4	0	20	8.4	8.0	19.9
0.002	0	15	0	75	8.5	7.9	20.0
0.004	5	16	25	80	8.4	7.9	20.0
0.008	7	18	35	90	8.4	7.9	19.9
0.02	10	20	50	100	8.4	7.9	19.9
0.04	17	20	85	100	8.4	7.9	19.9

[1] TS concentrations are based on nominal
*(20 organisms exposed in total per concentration)

Table A7_4_1_2(02)-7: Effect data

	EC_{50}[1]	95 % c.l.	EC_0[1]	EC_{100}[1]
24 h [mg a.i./l]	0.013	0.0071 – 0.023	0.002	>0.04
48 h [mg a.i./l]	0.0012	0.0008 – 0.0016	0.00033	0.019

[1] effect data are based on nominal (n) concentrations at 24 hours and mean measured concentrations at 48 hours.

Table A7_4_1_2(02)-8: Validity criteria for acute daphnia immobilisation test according to OECD Guideline 202

	fulfilled	Not fulfilled
Immobilisation of control animals <10%	**Yes**	
Control animals not staying at the surface	**Yes**	
Concentration of dissolved oxygen in all test vessels >3 mg/l	**Yes**	
Concentration of test substance ≥80% of initial concentration during test	**In part**	
Criteria for poorly soluble test substances	**Yes**	

4.3.3 Algal growth inhibition tests

4.3.3.1 Study 1 – Growth inhibition test on algae

Document IIIA/ Section A7.4.1.3/01	Growth inhibition test on algae
BPD Data Set IIA / Annex Point VII.7.3	

	Reference	**Official use only**
Data protection	*Yes*	
Data owner	*Bayer CropScience*	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	*Yes* *ISO-Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test) from 19.06.84 and OECD-Guideline No. 201 "OECD-Guideline*	

	for Testing of Chemicals", "Alga, Growth Inhibition Test" (07.06.84).	
GLP	*Yes*	
Deviations	None	
	Materials and Methods	
Test material	*NAK 4455 (transfluthrin technical)*	
Lot/Batch number	*130187*	X
Specification	As given in section 2	X
Purity	*95.0%*	X
Composition of Product	*N/A*	
Further relevant properties	*Appearance: brown liquid*	
Method of analysis	*Not stated*	
Preparation of TS solution for poorly soluble or volatile test substances	*See table A7_4_1_3(01)-1*	
Reference substance	*Yes, potassium dichromate (tested performed 11/02/1987).*	
Method of analysis for reference substance	*Not stated*	
Testing procedure		
Culture medium	*Mineral composition: (based on 1 litre) - 15 mg NH_4Cl, 12 mg $MgCl_2 \times 6H_20$, 18 mg $CaCl_2 \times 2H_20$, 15 mg $MGS0_4 \times 7H_20$, 1.6 mg KH_2P0_4, 80 µg $FeCl_3 \times 6H_20$, 100 µg $Na_2EDTA \times 2H_20$, 185 µg H_3BO_3, 415 µg $MnCl_2 \times 4H_20$, 3 µg $ZnCl_2$, 1.5 µg $CoCl_2 \times 6H_20$, 0.01 µg $CuCl_2 \times 2H_20$, 7 µg Na $Mo0_4 \times 2H_20$ and 50 mg $NaHCO_3$. pH: 8.19*	
Test organisms	*See table A7_4_1_3(01)-2*	
Test system	*See table A7_4_1_3(01)-3*	
Test conditions	*See table A7_4_1_3(01)-4*	
Duration of the test	*96 hours*	
Test parameter	*Cell multiplication inhibition*	
Sampling	*Cell counts were determined after 24, 48, 72 and 96 hours*	
Monitoring of TS concentration	*No*	
Statistics	*Cell count inhibition was determined using the following equation:*	
	$y = 0.00495\ x - 0.00000189\ x^2$	
	with $x = \frac{\text{cell count / ml}}{10^4}$ and y = extinction.	
	Results	
Preliminary test	*Performed*	
Concentration	*0.01 and 0.1 mg a.i./l (due to the low solubility of NAK 4455 (techn.) in water, the highest test concentration was 0.1 mg a.i./l). Control and solvent controls (0.1 ml/l) were also included in the study.*	
Preliminary Test results	*After 96 hours:*	

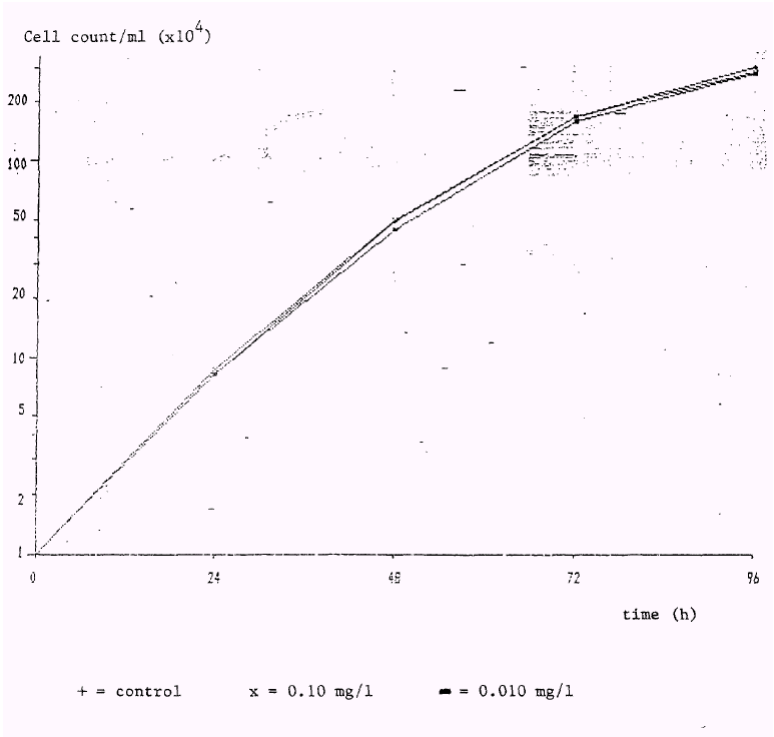
Concentration (mg a.i./l)	**cell counts (x 10^4/ml)*
Control	*301.17*
Solvent control	*298.73*
0.01	*276.52*
0.1	*284.35*

** mean value of 3 flasks*

Results test substance		
Initial concentrations of test substance	*Control, solvent control and 0.1 mg a.i./l.*	X
Actual concentrations of test	*Test reported in nominal concentrations.*	

substance

Growth curves



Concentration / response curve: *Not presented*

Cell concentration data: *See table A7_4_1_3(01)-5*

Effect data (cell multiplication inhibition): *See tables A7_4_1_3(01)-6 and A7_4_1_3(01)-7 for areas below the growth curve and growth rate effects.*

The EC_{50} of NAK 4455 determined for the biomass growth (E_bC_{50}) after 72 and 96 hours is > 0.1 mg a.i./l and the EC_{50} of the growth rate of the algae (E_rC_{50}) after 72 and 96 hours is also >0.1 mg a.i./l, based on nominal concentrations.

The "no-observed-effect-concentration" (NOEC) for the biomass and the growth rate is $\geq$0.1 mg a.i./l. No toxic effects were observed for biomass and growth rate even at the highest tested concentration of 0.1 mg a.i./l.

Other observed effects: *None*

Results of controls: *After 96 hours:*

Concentration (mg a.i./l)	**cell counts (x 10^4/ml)*
Control	*261.05*
Solvent control	*260.73*

** mean value of 3 flasks*

Test with reference substance: *Performed*

Concentrations: *0.18, 0.32, 0.56, 1.0, 1.80 mg/1*

Results: *An EC_{50} of 0.44 mg/1 was determined (based on biomass growth (E_bC_{50}) after 96 hours). This value is within acceptable limits (i.e. a ring test in which the test results varied from 0.20 to 0.75 mg/1).*

Applicant's Summary and conclusion

Materials and methods: *The study was conducted in accordance with ISO Guideline ISO/TC 147/SC 5/WG 5 N 84 (Algal Growth Inhibition Test), dated June 19, 1984 and the OECD Guideline No. 201 "OECD Guideline for Testing of*

	Chemicals", "Alga, Growth Inhibition Test" dated June 7, 1984. *The green algae* Scenedesmus subspicatus *was exposed to NAK 4455 for a period of 96 hours; under static conditions (at 23 ± 1°C and 8000 lux constant illumination) at a nominal concentration of 0.1 mg a.i./l. Control and solvent controls were also included in the study. After 24, 48, 72 and 96 hours cell counts were photometrically (at a wave length of 578 nm) determined in individual test vessels. In addition modifications of the cell structure were monitored; additional cell samples were taken at random from one flask at each of the treatment levels and the controls at each time-point and examined microscopically for abnormalities.* *Temperature and pH were monitored daily.*
Results and discussion	*No inhibition of cell biomass or growth rate was observed at the test concentration 0.1 mg a.i./l compared to the controls. No abnormalities such as alterations of the cell structure were observed.* *Water quality and environmental parameters were within acceptable limits.*
NOEC	*≥0.1 mg a.i./l.*
E_rC_{50}	*After 72 and 96 hours >0.1 mg a.i./l.*
E_bC_{50}	*After 72 and 96 hours >0.1 mg a.i./l.*
Conclusion	*The EC_{50} of NAK 4455 to* Scenedesmus subspicatus *was determined to be > 0.1 mg a.i./l for both biomass growth (E_bC_{50}) and growth rate (E_rC_{50}) after 72 and 96 hours. The "no-observed-effect-concentration" (NOEC) for the biomass and the growth rate is also ≥0.1 mg a.i./l. No toxic effects were observed for biomass and growth rate at the highest tested concentration of 0.1 mg a.i./l. Validity criteria were considered to be fulfilled.*
Reliability	*2*
Deficiencies	*Initial test concentration was not analytically confirmed, but this is not a stringent testing requirement.*

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	Evaluation by Rapporteur Member State
Date	*26-02-2007*
Materials and Methods	*Applicant's version is adopted with the following addition:* *3.1.1/3.1.2 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (95%), but this was most likely accounted for when preparing the test solution (concentrations in tables given as mg a.i./L). .*
Results and discussion	*Applicant's version is adopted.*
Conclusion	*Applicant's version is adopted.* *The results 96-hours $NOE_rC \geq 0.1$ mg/L and $E_rC_{50} > 0.1$ mg as/L are used for risk assessment.*
Reliability	*1*
Acceptability	*acceptable*
Remarks	*The highest test concentration of 0.1 mg/L is above the water solubility of 0.057 mg/L, but since the difference is < a factor of 2 and a solvent is used, the study is considered reliable.*
	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 A7_4_1_3(01)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	**Details**
Dispersion	*Yes, magnetic stirrer*
Vehicle	*Yes, solvent (acetone)*
Concentration of vehicle	*1.316 mg a.i./l stock, diluted 1:10 000 in water*
Vehicle control performed	*Yes, acetone control with 0.1 ml/L test solution*
Other procedures	*None*

Table A7_4_1_3(01)-2: Test organisms

Criteria	**Details**
Species	Scenedesmus subspicatus
Strain	*SAG 86/81*
Source	*Not stated*
Laboratory culture	*Yes*
Method of cultivation	*Stock cultures of algae were incubated at 16 hours illumination daily and 20° C in an autoclaved nutrient solution in accordance with BRINGMANN & KUHN (1980, Water Research 14: 231-241). Once a week the stock cultures are inoculated into fresh nutrient solution. All nutrient solutions are prepared with aseptically filtered, deionised water.*
Pretreatment	*Precultures were inoculated with 1 x 10^4 cells/ml 3 days prior to test start. Exponentially growing precultures were selected for the test.*
Initial cell concentration	*Approximately 1 x 10^4 cells/ml*

Table A7_4_1_3(01)-3: Test system

Criteria	**Details**
Volume of culture flasks	*300 ml with 100 ml test suspension*
Culturing apparatus	*Erlenmeyer flasks were exposed in a controlled-environment cabinet at 23±1°C and 8000 lux constant light.*
Light quality	*For illumination 2 x 4 fluorescent lamps (Osram L 140 W/20 Sa) were attached to the side of the controlled environment cabinet.*
Procedure for suspending algae	*Shaking*
Number of vessels/ concentration	*3*
Test performed in closed vessels due to significant volatility of TS	*Not specified*

Table A7_4_1_3(01)-4: Test conditions (main test only)

Criteria	**Details**

Test temperature	*22.5 – 22.7 ° C*
pH	*Start: 7.74 – 8.14* *End: 8.36 – 8.40*
Aeration of dilution water	*No*
Light intensity	*Approximately 8000 lux*
Photoperiod	*Constant light*

Table A7_4_1_3(01)-5: Cell concentration data (main study)

Test-Substance Concentration (nominal) [mg a.i./l]	**Cell concentrations (mean values) [x 10^4cells/ml]**									
	measured					**Percent of solvent control**				
	0 h	**24 h**	**48 h**	**72 h**	**96 h**	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	1.0	6.75	29.01	101.48	261.05	-	-	-	-	-
Solvent control	1.0	6.75	27.91	98.68	260.73	-	-	-	-	-
0.1	1.0	6.48	26.74	102.24	245.18	-	96	96	104	94

Table A7_4_1_3(01)-6: Area below the growth curves (main study)

Test-Substance Concentration (nominal) [mg a.i./l]	**Areas (A) below the growth curves**									
	measured					**Percent of solvent control**				
	0 h	**24 h**	**48 h**	**72 h**	**96 h**	**0 h**	**24 h**	**48 h**	**72 h**	**96 h**
Control	n.a.	69	474	2016	6342	-	-	-	-	-
Solvent control	n.a.	69	461	1956	6245	-	-	-	-	-
0.1	1.0	66	440	1964	6109	-	95.3	95.5	100.4	97.8

Table A7_4_1_3(01)-7: Growth rates and % deviation (main study)

Test-Substance Concentration (nominal) [mg a.i./l]	**Growth rates (μ)**				**Percent of Control**			
	0-24h	0-48h	0-72h	0-96h	0-24h	0-48h	0-72h	0-96h
Control	7.96	7.02	6.42	5.80	100.0	101.2	100.6	100.0
Solvent control	7.96	6.94	6.38	5.80	100.0	100.0	100.0	100.0
0.1	7.79	6.85	6.43	5.73	97.9	98.7	100.8	98.9

Table A7_4_1_3(01)-8: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	**Not fulfilled**
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**N.D.**	

N.D. Not determined (results based on nominal concentrations)

Criteria for poorly soluble test substances	**yes**	

4.3.3.2 Study 2 – Growth inhibition test on algae

Document IIIA/ Growth inhibition test on algae
Section A7.4.1.3/02
BPD Data Set IIA /
Annex Point VII.7.3

Reference

Official use

		only
Data protection	Yes	
Data owner	Bayer CropScience	
Companies with letters of access		
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 Guidelines and Quality Assurance	
Guideline study	Yes EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 3 'Algal inhibition test' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 201 'Alga, Growth Inhibition Test'.	
GLP	Yes	
Deviations	None Materials and Methods	
Test material	NAK 4455 (transfluthrin technical)	
Lot/Batch number	816779502	X
Specification	As given in section 2	X
Purity	95.7%	
Composition of Product	N/A	
Further relevant properties	Molecular weight: 371 g/mol Water solubility: 5.7 x 10^{-5} g/l Vapour pressure: 4.0 x 10^{-6} hPa (20°C)10^{-5}	
Method of analysis	Stability of test concentrations was verified by chemical analysis using Gas Chromatography (GC).	
Preparation of TS solution for poorly soluble or volatile test substances	See table A7_4_1_3(02)-1	
Reference substance	No	
Method of analysis for reference substance	N/A	
Testing procedure		
Culture medium	Mineral composition:	

Nutrient	Concⁿ in stock solution	Final concⁿ in the solution of the precultures & test cultures
$NaHCO_3$	solid	50 mg/L
Stock solution 1: macro-nutrients		
NH_4C1	1.5g/l	15 mg/1
$MgCl_2$ x 6 H_2O	1.2 g/1	12 mg/1
$CaCl_2$ x 2 H_2O	1.8 g/1	18 mg/1
$MgS0_4$ x 7 H_20	1.5 g/1	15 mg/1
KH_2PO_4	0.16 g/1	1.6 mg/I
Stock solution 2: Fe-EDTA		
$FeCl_3$ x 6 H_20	80 mg/l	80 µg/1
Na_2EDTA x 2 H_20	100 mg/l	100 µg/1
Stock solution 3: trace elements		
H_3B0_3	185 mg/l	185 µg/1
$MnCl_2$ x 4 H_20	415 mg/1	415 µg/1
$ZnCl_2$	3 mg/l	3 µg/1
$CoCl_2$ x 6 H_20	1.5 mg/1	1.5 µg/1
$CuCl_2$ x 2 H_20	0.01 mg/1	0.01 µg/1

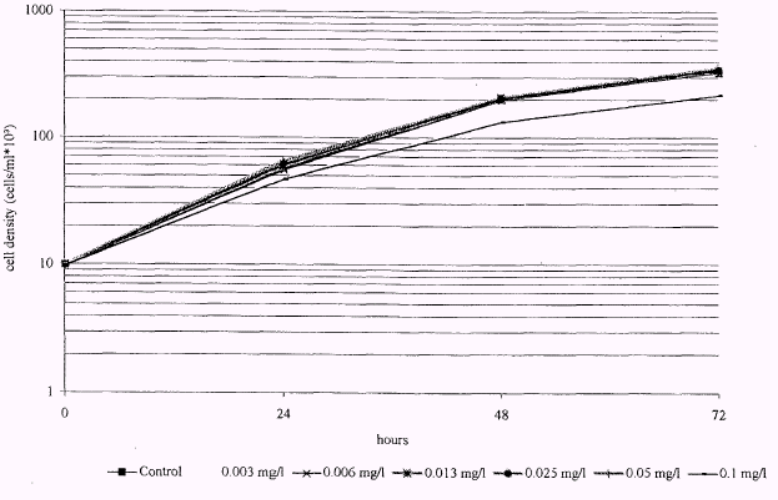
	Na_2Mo0_4 x 2 H_20	7 mg/1	7 µg/1	
Test organisms	See table A7_4_1_3(02)-2			
Test system	See table A7_4_1_3(02)-3			
Test conditions	See table A7_4_1_3(02)-4			
Duration of the test	72 hours			
Test parameter	Cell multiplication inhibition			
Sampling	Cell counts were determined after 24, 48 and 72 hours			
Monitoring of TS concentration	Yes, samples were taken at 0 and 72 hours and analysed using Gas Chromatography (GC).			
Statistics	Analysis of the growth (biomass) and growth rate of the algal population was performed using multiple t-Test according to Dunnett (1955).			
	Results			
Limit test	Not performed			
Concentration	N/A			
Number/ percentage of animals showing adverse effects	N/A			
Results test substance				
Initial concentrations of test substance	Control, 0.003, 0.006, 0.013, 0.025, 0.05 and 0.1 mg a.i./1, an additional sample of 0.1 mg a.i./l was prepared without algae to determine loss of substance due to algal uptake.			
Actual concentrations of test substance	Measured concentrations ranged from 50 - 80% of nominal values at 0 hours, and from 4.0-16.8% of nominal values at 72 hours, respectively. The highest test concentration was limited by the maximum water solubility of the test substance under exposure conditions.			X

Test concentration (mg a.i/l)	0 hours	72 hours
Control	<0.001	<0.001
0.003	0.002	<0.001*
0.006	0.003	<0.001*
0.013	0.007	0.001
0.025	0.015	0.001
0.05	0.031	0.003
0.1	0.080	0.007
0.1 (no algae)	0.081	0.075

*Values below the quantitation limit (0.001mg/1) regarded as 0.0005 mg/1, i.e. half of the quantitation limit.

The measured concentration in the parallel sample of 0.1 mg a.i./L incubated without algae revealed 81 to 75% of the initial concentration.

Document IIIA/ Section A7.4.1.3/02 BPD Data Set IIA / Annex Point VII.7.3	**Growth inhibition test on algae**

Growth curves		
Concentration / response curve	Not presented	
Cell concentration data	See table A7_4_1_3(02)-5	
Effect data (cell multiplication inhibition)	Based on mean measured concentrations the EC_{50} of NAK 4455 determined for the biomass growth (E_bC_{50}) after 72 hours is > 0.044 mg a.i./l and the EC_{50} of the growth rate of the algae (E_rC_{50}) after 72 hours is also >0.044 mg a.i./l. The "no-observed-effect-concentration" (NOEC) for the biomass and the growth rate is 0.017 mg a.i./l.	X
Other observed effects	The test compound disappeared faster from test vessels including algae. This suggests uptake of the a.i. and hence, significant exposure.	
Results of controls		

Concentration (mg a.i./l)	*cell density (cells/ml) (initial cell density = 10^4 cells/ml		
	24 h	48 h	72h
Control	58333	210556	336667

* mean value of 6 replicates

Test with reference substance	Not performed
Concentrations	N/A
Results	N/A
	Applicant's Summary and conclusion
Materials and methods	The study was conducted in accordance with EEC Methods for Determination of Ecotoxicity Annex to Directive 92/69/EEC (O.J. No. L383A, 29.12.92) Part C, Method 3 'Algal inhibition test' which is in most parts equivalent to the OECD Guideline for Testing of Chemicals No. 201 'Alga, Growth Inhibition Test'. Dates of experimental work: 05/06/2001 to 08/06/2001. The green algae Scenedesmus subspicatus was exposed to NAK 4455 for a period of 72 hours; under static conditions (at 23 ± 2°C and 6000 - 10000 lux constant illumination) at nominal concentrations of 0.003, 0.006, 0.013, 0.025, 0.05 and 0.1 mg a.i./l. Controls for analysis of the stability of the test substance as well as effects without treatment were also included in the study. After 24, 48 and 72 hours cell densities were measured in a microcell counter or alternatively by means of a microscopic counting chamber. Temperature and pH were measured at the start and end of the study.

Document IIIA/ Section A7.4.1.3/02 BPD Data Set IIA / Annex Point VII.7.3	**Growth inhibition test on algae**	
	Analytical samples were taken from the controls and each test concentration at 0 and 72 hours and analysed using GC.	
Results and discussion	Significant inhibition of cell multiplication (66%), cell biomass (34.4%) and growth rate (11.8 %) was observed after 72 hours at a nominal concentration of 0.1 mg a.i./l (compared to the controls). No negative effects were observed at the remaining test concentrations, only 1.1% inhibition was observed at 0.006 mg/L. The 72-hour E_rC_{50} and E_bC_{50} were calculated to be >0.044 mg a.i./l (based on mean measured concentrations). Measured concentrations ranged from 50 - 80% of nominal values at 0 hours, and from 4.0-16.8% of nominal values at 72 hours, respectively in test vessels containing algal suspensions The highest test concentration was limited by the maximum water solubility of the test substance under exposure conditions. The analysis of the test concentration of 0.1 mg a.i./L, incubated in parallel without algae showed 81% and 75% of the nominal concentration at 0 and 72h, respectively. Water quality and environmental parameters were within acceptable limits.	
NOEC/LOEC	0.017 mg a.i./L and 0.044 mg a.i./l (based on mean measured concentrations), respectively	X
E_rC_{50}	After 72 hours >0.044 mg a.i./l (based on mean measured concentrations).	X
E_bC_{50}	After 72 hours >0.044 mg a.i./l (based on mean measured concentrations).	X
Conclusion	The EC_{50} of NAK 4455 to Scenedesmus subspicatus was determined to be > 0.044 mg a.i./l for both biomass growth (E_bC_{50}) and growth rate (E_rC_{50}) after 72 hours. The "no-observed-effect-concentration" (NOEC) for the biomass and the growth rate was determined to be 0.017 mg a.i./l.	X
Reliability	1	
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	26-02-2007
Materials and Methods	Applicant's version is adopted with the following addition: 3.1.1/3.1.2 Batch differs from those included in the batch analysis (Doc III A.2 confidential). Purity of test substance is low (95.7%), but concentrations were measured. 3.4.8 For the analytical measurements, only the resulting figures are given. Method and results are not described in detail (i.e. it is not clear whether samples were analysed in duplicate, recovery is given as 96 %, but spiking level and individual data are not given).

Results and discussion	*Applicant's version adequately reflects the report. The following comments can be made:* *4.2.2 Actual concentrations at t = 0 and t = 72 hours were 50 – 80 and 4.0 – 17 %, respectively. In the medium without algae, recovery was 81 and 75 % of nominal after 0 and 72 hours, indicating that measurement in the other test concentrations were highly influenced by the presence of the algae. This is not unexpected in view of the strong sorptive characteristics of transfluthrin. Since the average measured concentration in the medium without algae is close to 80 %, it is considered justified to evaluate the effects on the basis of nominal concentrations.* *5.2 The 72-hours E_rC_{50} based on nominal concentrations is > 0.1 mg/L.*
Conclusion	*The results 72-hours NOE_rC 0.05 mg/L and E_rC_{50} > 0.1 mg/L (nominal) are used for risk assessment*
Reliability	*2* *The highest test concentration is above the reported water solubility of 0.057 mg/L, recovery in test media without algae was slightly lower than 80 %.*
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 A7_4_1_3(02)-1: Preparation of TS solution for poorly soluble or volatile test substances

Criteria	Details
Dispersion	*Yes, A stock solution was prepared to give the desired series of test concentrations. 0.9 mg of the test substance was added to 2 litres of dilution water and treated for 60 seconds with an ultra turrax and afterwards stirred for 24 h on a magnetic stirrer. Finally undissolved particles of the test substance were removed by filtration.*
Vehicle	*No (dilution water only)*
Concentration of vehicle	*N/A*
Vehicle control performed	*N/A*
Other procedures	*None*

Table A7_4_1_3(02)-2: Test organisms

Criteria	Details
Species	Scenedesmus subspicatus *CHODAT*
Strain	*Non-axenic strain*
Source	*The Collection of Algal Cultures' of the Institute of Plant Physiology at the University of Gottingen (Germany)*
Laboratory culture	*Yes*
Method of cultivation	*Exponentially-growing stock cultures are maintained in the test facility under constant temperature*

	conditions (23 ± 2°C) at a light intensity in the range 60 – 120 µE. x m^{-2} x s^{-1} (measured in the range 400 to 700 nm using a spherical quantum flux meter). The nutrient medium (according to BRINGMANN & KÜHN; 1977) is renewed once a week. Cell density measurements are made using a microcell counter.
Pretreatment	*Pre-cultures are set up three days before the start of a test. They are grown under identical exposure conditions as the stock cultures, except from the use of a different nutrient medium.*
Initial cell concentration	*Approximately 1 x 10^4 cells/ml*

Table A7_4_1_3(02)-3: Test system

Criteria	**Details**
Volume of culture flasks	*300 ml*
Culturing apparatus	*Erlenmeyer flasks were exposed in a light chamber in which a temperature in the range 21 °C to 25°C can be maintained at ± 2°C, and continuous uniform illumination is provided in the spectral range 400 to 700 nm.*
Light quality	*A light intensity in the range 60 to 120 µE. x m^{-2} x s^{-1}, or an equivalent range of 6000 to 10000 lux, is recommended for use. Light source not stated.*
Procedure for suspending algae	*Not stated*
Number of vessels/ concentration	*3 replicates per test concentration and 6 replicates per control*
Test performed in closed vessels due to significant volatility of TS	*Yes, stoppers*

Table A7_4_1_3(02)-4: Test conditions (main test only)

Criteria	**Details**
Test temperature	*23 ± 2° C*
pH	*Start: 7.7 – 8.3* *End: 9.4 – 10.4*
Aeration of dilution water	*No*
Light intensity	*Approximately 6000 - 10000 lux*
Photoperiod	*Constant light*

Table A7_4_1_3(02)-5: Cell concentration data

Test-Substance Concentration (nominal)[1] [mg a.i./l]	**Cell concentrations (mean values) [cells/ml]**							
	measured				**Percent of control**			
	0 h	**24 h**	**48 h**	**72 h**	**0 h**	**24 h**	**48 h**	**72 h**
Control	1000	58333	210556	336667	-	-	-	-
0.003	1000	63333	201111	350000	-	109	96	104
0.006	1000	56667	204444	343333	-	97	97	102
0.013	1000	60000	210000	344444	-	103	99	102
0.025	1000	61111	205556	350000	-	105	98	104
0.05	1000	65556	210000	361111	-	112	99	107
0.1	1000	47778	136667	222222	-	82	65	66

[1] TS concentrations were nominal

Table A7_4_1_3(02)-6: Growth (biomass) and growth rate of algae

Test-Substance Concentration	**Growth (b)**	**Percent inhibition**	**Growth rate (r)**	**Percent inhibition**

(nominal)[1] [mg a.i./l]		of Control (b)*		of Control (r)
Control	412222	0.0	1.17	0.0
0.003	414444	-0.5	1.19	-1.1
0.006	407778	1.1	1.18	-0.6
0.013	417222	-1.2	1.18	-0.6
0.025	416667	-1.1	1.19	-1.1
0.05	431111	-4.6	1.20	-2.0
0.1	270556	34.4	1.03	11.8

(b), biomass, (r), growth rate
* negative values indicate growth greater than control algae

Table A7_4_1_3(02)-7: Validity criteria for algal growth inhibition test according to OECD Guideline 201

	fulfilled	Not fulfilled
Cell concentration in control cultures increased at least by a factor of 16 within 3 days	**yes**	
Concentration of test substance ≥80% of initial concentration during test	**No***	
Criteria for poorly soluble test substances	**yes**	

* Results based on mean measured concentrations

4.3.4 *Lemna* sp. growth inhibition test

No data available.

4.4 Chronic toxicity

IUCLID IIIA 9.1.6. BPD Data Set IIA / Annex Point VII.7.3 **fish early-life stage toxicity**

		Official use only
Data protection	Data protection claimed	
Data owner	Data submitter is data owner	
Guideline study	OECD Guideline 210 (Fish, Early-Life Stage Toxicity Test)	
GLP	Yes	
Deviations	-	
Test material	Transflutrin technical	
Lot/Batch number	PNLS000112	
Specification	102000008838-3	
Purity	97.7 %	
Analytical monitoring	yes	
Details on sampling	Two replicates were alternately sampled at each interval (weekly). 1000 ml of water were sampled at mid-depth in each aquarium. Samples were not stored before analysis.	
Details on analytical methods	DETAILS ON PRETREATMENT - Extraction : Liquid-liquid extraction. three times with 65 mL of methylene chloride. The combined bottom phases were dried at approximately ≤35°C ±2 ºC on a turbo vap LV or equivalent using nitrogen. The results were dissolved in a measured quantity of ethyl acetate. IDENTIFICATION AND QUANTIFICATION OF TEST SUBSTANCE/PRODUCT - Separation method: GC-MS/MS	

	- Conditions: Agilent DB-5MS; 12 meter x 0.20 mm x 0.33 µm - Detection method: GC-MS/MS - Detection limits: LOQ 0.5 ng a.i./L - Reproducibility in % (indicate method of evaluation; should be given for stated concentration levels): - Linearity range: 4E-4 to 2E-2 µg/mL - Calibration: external - Extraction recovery (indicate if results are corrected or not for recoveries):
Vehicle	Yes
Details on test Solutions	A 36-day preliminary range-finding study was conducted under flow-through conditions. Nominal concentrations were control, solvent control, 4.00, 20.0, 100, and 500 ng a.i./L. No treatment related effects were determined in any test level as compared to the controls. Results from this range-finding test were used to define the lowest tested concentrations in the definitive test. Read-across with results from independent GLP acute studies with the golden orfe and the rainbow trout exposed to Transfluthrin technical were used to define the highest tested concentration of 1000 ng a.i./L. Solvent : Dimethylformamide (DMF) CAS# 68-12-2, 10 µl/L
Test organisms (species)	Pimephales promelas
Details on test organisms	TEST ORGANISM - Common name: Fathead minnow (Pimephales promelas) - Parental Lot Number: EG031914 - Source: In-house METHOD FOR PREPARATION AND COLLECTION OF FERTILIZED EGGS <24 hours old eggs were removed from breeding substrates on the morning of the experimental start and nonviable eggs were discarded. Eggs appropriate for testing were pooled for impartial placement of eggs into randomly positioned oscillating egg cups. POST-HATCH FEEDING - Start date: Day 4 (thinning day) - Type/source of feed: Brine shrimp (Artemia salina) - Amount given: Equal distribution at 0.2 - 2.0 ml per feeding (feeding rate was increased as fish grew during the exposure) - Frequency of feeding: One to three times daily until approximately 24 hours prior to study termination
Test type	flow-through
Water media type	freshwater
Limit test	no
Total exposure duration	36.0 d
Hardness	42 to 54 mg/L as CaCO3
Test temperature	23.8 to 25.8 °C
pH	7.2 to 8.1
Dissolved oxygen	D.O. Range: 2 to 8.0 mg/L (87 to 97% saturation) Mean: 7.6 mg/L (92% saturation)
Nominal and measured concentrations	Nominal (arithmetic mean measured) concentrations : - 62.5 (28.0) ng a.i./L - 125 (53.0) ng a.i./L - 250 (95.0) ng a.i./L - 500 (190) ng a.i./L - 1000 (399) ng a.i./L
Details on test conditions	TEST SYSTEM

- Embryo cups: Oscillating egg cup
- Test vessel: all glass
- Type (delete if not applicable): open
- Material, size, headspace, fill volume: 1-L modified proportional diluter system, One Hamilton
- Microlab 600 Series pump, Hamilton Microlab 500 Series pump, Splitter cups, mixing chambers, fill
- volume: 7L, Headspace: 1.4L, vessel size: 21.6 x 12.7 x 30.5 cm
- Aeration: No
- Type of flow-through (e.g. peristaltic or proportional diluter): Proportional diluter
- Renewal rate of test solution (frequency/flow rate): 12 turnovers/vessel/24h
- No. of fertilized eggs/embryos per vessel: 35 eggs/ 20 alevins after hatching phase
- No. of vessels per concentration (replicates):4
- No. of vessels per control (replicates):4
- No. of vessels per vehicle control (replicates):4
- Biomass loading rate: 0.0364 g/L during a 24-hour period

TEST MEDIUM / WATER PARAMETERS

- Source/preparation of dilution water: dechlorinated municipal water blended with reverse osmosis
- water designed to produce soft (40 to 60 mg/L as CaCO3) water, intensely aerated, sterilized through
- ultraviolet, stored in polypropylene or PVC holding tanks.
- Total organic carbon: 1.2 µg/L
- Alkalinity: 20 to 31 mg/L as CaCO3
- Conductivity: 159.5 to 198.8 µmhos/cm
- Culture medium different from test medium: No
- Intervals of water quality measurement: weekly

OTHER TEST CONDITIONS

- Adjustment of pH: No
- Photoperiod:16 hours light/ 8 hours dark (with 30 minute dawn/dusk transition period)
- Light intensity: 788 – 891 lux (mean 831 lux)
- EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : abnormal behavior
- (daily), physical changes (daily), mortality(daily) and growth (at the end of exposure)

VEHICLE CONTROL PERFORMED: yes

RANGE-FINDING STUDY

- Test concentrations: control, solvent control, 4.00, 20.0, 100, and 500 ng a.i./L
- Results used to determine the conditions for the definitive study: Yes (lowest tested concentrations)

POST-HATCH DETAILS

- Begin of post-hatch period: After thinning
- No. of hatched eggs (alevins)/treatment released to the test chamber: 20
- Release of alevins from incubation cups to test chamber on day no.: Day 4

FERTILIZATION SUCCESS STUDY

- Number of eggs used:
- Removal of eggs to check the embryonic development on day no.:

Overall remarks

All biological validity criteria for this study were met. The average

	hatchability and the average post hatch survival of controls were >80%. Each control replicate had at least 70 percent survival. Dissolved oxygen was > 60%. The discrepancy of measured and nominal values can be explained by the physico-chemical properties of Transfluthrin which cause fast dissipation from the water even under flow-through conditions. The ecotoxicological endpoints were calculated based on mean measured concentrations.
Validity criteria fulfilled	yes
Conclusions	None of the observed endpoints (hatching rate, mortality, growth in length and weight, behaviour and fish morphology) were impaired by the test compound. The 36-day exposure to Transfluthrin Technical resulted in an overall NOEC of 389 and a LOEC of > 399 ng a.i./L.
Executive summary	The 36 -day chronic toxicity of Transfkuthrin to early life stage of Fathead minnow (Pimephales promelas) has been studied under flow throw conditions. Fertilized eggs <24 hours old were exposed to blank, vehicle blank, and to test material with nominal concentrations (mean measured) of: 62.5 (28.0), 125 (53.0), 250 (95.0), 500 (190), and 1000 (399) ng a.i./L. The system was maintained at a temperature range of 23.8 to 25.8 and a pH of 7.2 to 8.1 . The hatching phase started at Day 0 and ended at Day 4. The 36-day exposure to Transfluthrin Technical resulted in an overall NOEC of 389 and a LOEC of > 399 ng a.i./L.
	Evaluation by Competent Authorities
Remarks	All validity criteria required were fulfilled: - The average hatchability and the average post hatch survival of controls were >80% (> 90%) - Each control replicate had at least 70 percent survival (> 90 %) - Dissolved oxygen was > 60 % (87-97 %) All environmental parameters were within the ranges defined by the guidelines OCSPP 850.1400 and OECD 221. The analytical section of the study report presents the representative chromatograms, storage conditions, storage time of the samples transfluthrin taken and results of the calibration standards (including calibration curve). The RMS considers the study reliable without restriction. 36 d NOEC 399 ng/L (mean measured)

4.4.1 Fish early-life stage (FELS) toxicity test

No data available.

4.4.2 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.3 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.4 Chronic toxicity to aquatic invertebrates

	Reference	**Official use only**
Data protection	yes	
Data owner	data submitter is data owner	
Guideline study	OECD Guideline 211 (Daphnia magna Reproduction Test)	
GLP	yes	
Deviations	yes Light intensity : between 512 and 746 lux during light periods	
Test material	Transfluthrin Technical	
Lot/Batch number	PNLS000112	
Specification		
Purity	97.7%	
Analytical monitoring	yes	
Details on sampling	Two replicates were alternately sampled at each interval (weekly). Approximately 1000 ml of test wa ter were sampled for analysis. Samples were not stored before analysis.	
Details on analytical methods	DETAILS ON PRETREATMENT - Extraction (solvent used, method: e.g. liquid-liquid, SPE): Liquid-liquid extraction. three times with 65 mL of methylene chloride. The combined bottom phases were dried at approximately <35°C ±2 °C o n a turbo vap LV or equivalent using nitrogen. The results were dissolved in a measured quantity of ethyl acetate. IDENTIFICATION AND QUANTIFICATION OF TEST SUBSTANCE/PRODUCT - Separation method (e.g. HPLC, GC): GC-MS/MS - Conditions (column, mobile phase, etc.): Agilent DB-5MS; 12 meter x 0.20 mm x 0.33 ^m - Detection method (e.g. ECD, UV, MS, ICP-AES, ICP-MS): GC-MS/MS - Detection limits (LOD, LOQ) (indicate method of determination/calculation): 1.0 ppt, 1.0 ng a.i./L - Reproducibility in % (indicate method of evaluation; should be given for stated concentration levels): - Linearity range: 8E-5 to 2E-2 ^g/mL - Internal or external calibration: external - Extraction recovery (indicate if results are corrected or not for recoveries):	
Vehicle	yes	
Details on test Solutions	A 21-days preliminary range-finding study was conducted under flow-though conditions Nominal concentrations were control, solvent control, 2.40, 12.0, 60.0, and 300 ng a.i./L. This information was used to set definitive test levels. Solvent : Dimethylformamide (DMF) CAS# 68-12-2 (10 ^l/L)	
Test organisms (species)	Daphnia magna	
Details on test organisms	- First instar < 24 hours old neonates from third brood or older, at initiation TEST ORGANISM - Common name: Daphnia magna - Strain/clone: neonates from third brood or older - Source: Aquatic Biosystems, Fort Collins, CO - Age of parental stock (mean and range, SD): First instar < 24 hours old - Feeding during test : yes	

	- Food type: combination of Green algae (Pseudokirchneriella subcapitata) and blended Tetrafin flaked fish food - Amount: > 2.0 x 108 algal cells/L and Tetrafin suspension (5 mg/mL) at increasing rates throughout the study - Frequency: Algae was fed daily and Tetrafin was fed on three days each week.
Test type	flow-through
Water media type	freshwater
Limit test	no
Total exposure duration	21.0 d
Hardness	166 mg/L as CaCO3
Test temperature	20.1 to 20.4 °C
pH	7.8 to 8.2
Dissolved oxygen	8.2 to 8.4 mg/L (90 to 92% saturation)
Nominal and measured concentrations	Nominal (mean measured) ng a.i./L: - 7.00 (4.02) - 14.0 (8.85) - 28.0 (17.5), - 56.0 (35.7) - 112 (68.6)
Details on test conditions	TEST SYSTEM - Test vessel: 400 ml beakers containing approximately 300 ml water - Aeration: No - Type of flow-through (e.g. peristaltic or proportional diluter): diluter system used for intermittent delivery of test solutions to test chambers. Splitter cups were used to equally divide (±10%) test solutions between replicates at each level - Renewal rate of test solution (frequency/flow rate): 6 Turnover/Vessel/24 hours - No. of organisms per vessel: 5 - No. of vessels per concentration (replicates): 4 - No. of vessels per control (replicates): 4 - No. of vessels per vehicle control (replicates): 4 TEST MEDIUM / WATER PARAMETERS - Source/preparation of dilution water: spring water blended with reverse osmosis water designed to produce hard (160 to 180 mg/L as CaCO3) water. The spring water is collected from a spring box, passed through a multimedia filter, a 5-micron bag filter, granular activated carbon filters, a 1-micron cartridge filter and finally an ultraviolet sterilizer. Dilution water is stored in polypropylene or PVC ho lding tanks and intensely aerated before use OTHER TEST CONDITIONS - Adjustment of pH: No - Photoperiod: 16 hours light, 8 hours dark - Light intensity: 512 to 746 lux (measured range) EFFECT PARAMETERS MEASURED (with observation intervals if applicable) : - Survival at Termination : daily - Time to First Brood - Neonates per Adult Reproduction Day : at the time of first brood release and on Monday, Wednesd ay and Friday thereafter (to include the day of termination) - -Living Neonates Produced per Adult : at the time of first brood

	release and on Monday, Wednesday and Friday thereafter (to include the day of termination) - Adult Body Length : at the end of the exposure - Adult Dry Weight : at the end of the exposure VEHICLE CONTROL PERFORMED: yes RANGE-FINDING STUDY - Test concentrations: - Results used to determine the conditions for the definitive study:
Reference substance (positive control)	no
Results	At test termination, on study day 21, traces of Transfluthrin (i.e. 1.4 ng a.i./L which is around the LOQ) were detected in the control and solvent control samples. Since the dosing system was still running the next day, additional samples were taken from the control and the solvent control one day post termination. No Transfluthrin was detected in these samples, which suggests that the flow-through system functioned correctly. The contamination on day 21 can be explained by cross contamination during the sampling process. Two possible scenarios have been identified. 1. Sampling with a contaminated beaker. 2. Contamination occurring during the collection of organisms for length measurements. The study is considered valid due to the fact that this contamination occurred only on one sampling event, which was the last day of the experiment and that the concentration was very low (around the LOQ).
Overall remarks	All biological validity criteria for this study were met. Mean parental survival of the control and solvent control daphnids was 85%. Time to first brood in both the control and solvent control group was day 8 for all replicates. The number of neonates per parent at the start of the test in all replicates of the control and solvent control was > 60.
	Applicant's summary and conclusion
Validity criteria fulfilled	yes
Conclusions	The NOEC and LOEC were calculated based on mean measured concentrations. The 21-day exposure to Transfluthrin technical resulted in a NOEC of 17.5 ng a.i./L and a LOEC of 35.7 ng a. i./L based on the number of neonates per adult reproduction day. The lowest EC10 and associated 95% confidence limits was calculated to be 18.3 (12.8 to 55.9) ng a.i./L for the endpoint of adult dry weight.
Executive summary	In a 21-day chronic test first instars of Daphnia magna (<24 hours old) were exposed to nominal (mean measured) concentrations of control (<1.0), solvent control (<1.0), 7.00 (4.02), 14.0 (8.85), 28.0 (17.5), 56.0 (35.7) and 112 (68.6) ng a.i./L for 21 days under flow-through conditions. Arithmetic mean concentrations are based on recoveries from Days 0, 7, 14, and 21. Sublethal effects, survival (immobilization), time to first brood release, reproduction (living neonates per adult at start of the study, neonates per adult reproductive day) and growth (length and dry weight at study termination) were recorded. The 21 -day NOEC was 17.5 ng a.i./L and LOEC was 35.7 ng a.i./L based on the number of neonates per adult reproduction day. The lowest EC10 and associated 95% confidence limits was calculated to be 18.3 (12.8 to 55.9) ng a.i./L for the endpoint of adult dry weight. This study is classified as acceptable with restrictions for a long term toxicity study with freshwater invertebrates. Results Synopsis Test Organism Age : below 24 h Test Type :flow-through

IUCLID IIIA 9.1.6.2 BPD Data Set IIA / Annex Point VII.7.3	long-term toxicity to aquatic invertebrates
	21 days-NOEC =17.5 ng a.i./L 21 days-LOEC =35.7 ng a.i./L Endpoint(s) Effected: reproduction
	Evaluation by Competent Authorities
Remarks	All validity criteria required were fulfilled: - Mean parental survival of the control and solvent control daphnids was > 80% (85%). - Time to first brood in both the control and solvent control group was day 8 for all replicates. - The number of neonates per parent at the start of the test in all replicates of the control and - solvent control was > 60. All environmental parameters were within the ranges defined by the guidelines OCSPP Guideline 850.1300 and OECD 211. The analytical section of the study report presents the representative chromatograms, storage conditions, storage time of the samples transfluthrin taken and results of the calibration standards (including calibration curve). The RMS considers the study reliable without restriction. 21 d NOEC 17.5 ng/L (mean measured)

4.4.5 Chronic toxicity to algae or aquatic plants

See short-term toxicity.

4.5 Acute and/or chronic toxicity to other aquatic organisms

Not available.