Bayer CropScience

Document Title

Summary of the toxicological and metabolism studies for Fluoxastrobin

Data Requirements

EU Regulation 1107/2009 & EU Regulation 283/2013

Document MCA

Section 5: Toxicological and metabolism studies

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

Date

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Bayer CropScience

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Version history

Date	Data points containing amendments or additions[1] and brief description	Document identifier and version number
2016-01-13	Original submission	M-544192-01-1
2016-03-10	Update of document to include reference to additional data requested by RMS UK at admissibility check (M-549514-01-1: p. 30, p. 68 and p. 74)	M-544192-02-1

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

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CA 5 TOXICOLOGICAL AND METABOLISM STUDIES ON THE ACTIVE SUBSTANCE

As published in Commission Directive 2008/44/EC of 04th April 2008 and with an Entry into Force (EIF) date of 01st August 2008, the fungicide Fluoxastrobin was first included in Annex I to Commission Directive 91/414/EEC.

Now, with the aim to achieve European Re-Approval under Regulation 1107/2009, Bayer CropScience (BCS) provides this '**Supplementary Dossier**'. It contains only new data which were not submitted at the time of the Annex I inclusion of fluoxastrobin under Commission Directive 91/414/EEC and which were therefore not evaluated during the first European review.

In addition to submitting the above mentioned Supplementary Dossier, all studies relied upon under 91/414 and contained in the Draft Assessment Report and its Addenda are – for the convenience of the reviewers – included in what BCS calls '**Baseline Dossier**' (Document K level only).

In order to ease the reviewers' orientation on 'old' studies in the Baseline Dossier versus 'new' studies in the Supplementary Dossier, BCS has decided to apply the following basic principles:

1. Conversion of the Document K part of the old EU dossier structure into the new structure (acc. to Commission Regulations 283/2013 and 284/2013 and linking the old studies to the new structure according to the cross-walk tables provided in Guidance Document SANCO/10181/2013 – rev. 2.1 of 13th May 2013.
2. On a case-by-case basis and where useful for the reader, old studies from the Baseline Dossier are occasionally summarised on the Document M level of the Supplementary Dossier; the text of those summaries is formatted in grey font colour. However, where useful additional information is occasionally given either in the summary text or summary tables in black font.
3. For any referenced old study, its bibliographic information (e.g. author, year, document number) is formatted in grey font colour.
4. For any new study, its bibliographic information and its free flow summary text and table content is formatted in standard black font colour.

Where applicable, the above formatting rules above apply to all dossier elements (e.g. MCA, MCP, JCA etc.).

According to the guidance of EFSA on the "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009" (EFSA Journal 2011; 9(2):2092), literature for the active substance and its metabolites needs to be presented, covering the last 10 years prior to the submission of this Annex I renewal dossier. In relation to this section 5 no adequate scientific peer-reviewed open literature was identified which would need to be scientifically considered. There were no findings in the scientific peer-reviewed open literature for the active substance fluoxastrobin and its metabolites which might have a possible impact on an end-point or the risk assessments.

For substance codes, synonyms and abbreviations please refer to '*Document N3 - 'Substances and metabolites: structure, codes, synonyms – Fluoxastrobin*'.

Note: Denomination of the active substance and its isomers

In the original reports the active substance and the E-and Z-isomer are sometimes denominated differently. Initially the common name fluoxastrobin (chemical code HEC 5725) was assigned to both, the E-and Z-isomer as a sum and thus in some reports the active substance fluoxastrobin is used as a

synonym for both isomers as a sum. During the EU peer review it was agreed to define the active substance fluoxastrobin as the E-isomer only and the Z-isomer was assigned as an impurity. The definition of the active substance is laid down in the EFSA Scientific Report (2007) 102, 1-84 and in the Inclusion Directive 2008/44/EC (4 April 2008).

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

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

The scientific information of the studies in Table 5.1.1-1 and Table 5.1.1-2 was evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008).

Table 5.1.1-1: ADME experiments conducted with radiolabelled fluoxastrobin in rats

Type of experiment	Dose* (mg/kg bw)	Test animals		Radiolabel	Reference/report
		Sex	No.		
Expiration, single low dose	1	male	4	[chlorophenyl-UL-^{14}C]	[redacted],; 2002; M-041524-01-1
	1	male	4	[pyrimidine-2-^{14}C]	[redacted] R; 2001; M-033650-01-1
	1	male	4	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted],; 2001; M-033929-01-1
Single low dose	1	male	4	[chlorophenyl-UL-^{14}C]	[redacted],; 2002; M-041524-01-1
	[illegible]	male	4	[pyrimidine-2-^{14}C]	[redacted],; 2001; M-033650-01-1
	1	male & female	4 & 4	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted]; 2001; M-033929-01-1
Single high dose	100	male & female	4 & 4	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted],; 2001; M-033929-01-1
Repeated low dose#	1 (14x + 1x)#	male & female	4 & 4	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted] V; 2001; M-033929-01-1
Bile-duct cannulation, single low dose	1	male	6	[chlorophenyl-UL-^{14}C]	[redacted] U; 2002; M-041524-01-1
	1	male	6	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted],; 2001; M-033929-01-1
Whole body autoradiography, single low dose, prior collection of urine and faeces		male & female	8 & 8	[chlorophenyl-UL-^{14}C]	[redacted],; 2002; M-041524-01-1
	3	male & female	8 & 8	[pyrimidine-2-^{14}C]	[redacted],; 2001; M-033650-01-1
	3	male & female	5 & 5	[methoxyiminotolyl-ring-UL-^{14}C]	[redacted] K; 2001; M-033929-01-1

* dosed orally as a suspension in 0.5% aqueous Tragacanth

\# 14 daily doses with non-labelled fluoxastrobin and another last dose with radiolabelled fluoxastrobin

Table 5.1.1-2: ADME experiments conducted with the radiolabelled metabolite 2-chlorophenol (M82) in rats

Type of experiment	Dose* (mg/kg bw)	Test animals		Radiolabel	Reference/report
		Sex	No.		
Single low dose including expiration	5	male	4	[phenyl-UL-^{14}C]	[redacted]; 2002; M-041282-01-1

* dosed orally as a 0.9% sodium chloride solution in water

Conclusion from the EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised 13 June 2007":

Fluoxastrobin is rapidly and nearly completely absorbed from the gastrointestinal tract (80 - 92% of administered dose of 1 mg/kg bw within 24 - 30 hours post administration). It is widely distributed within the body of the treated animals at generally low concentrations. The highest concentrations are detected in liver, kidneys and bladder as well as in the gastrointestinal tract. No indication of significant accumulation in the body is observed. Excretion of fluoxastrobin related residues occurred fast and at a high rate. The major route of excretion in rats was biliary and correspondingly faecal (84-100% within 48 hours, mostly via bile). Fluoxastrobin is extensively metabolised and 24 hours post dose the portion of unchanged parent compound is < 10% of the administered radioactivity). The metabolic pattern is complex and 50 metabolites are identified. Only a few metabolites were found to be prominent: they were hydroxylated metabolites, which still contained all 3 rings, notably M12 and M25, as well as HEC 5725-E-des-chlorophenyl (M48) and HEC 5725-des-chlorophenyl-dioxazine-OH (M49). Metabolism in rats is qualitatively similar to that in goats and hens.

Comparative in vitro metabolism studies

For comparative in-vitro metabolism studies, test methods or guidance documents published in form of an update of the Commission Communications 2013/C 95/01 and 2013/C 95/02 are not yet available. According to point 4 (Documents to be included in a submission) of the current version of the Guidance Document for Applicants on Preparing Dossiers for the Approval of a Chemical New Active Substance and of the Renewal of Approval of a Chemical Active Substance According to Regulation (EU) No 283/2013 and Regulation (EU) No 284/2013 (SANCO/10181/2013), waiving of these particular data requirement points is considered acceptable and is requested hereby.

For Fluoxastrobin, no comparative in-vitro metabolism study was conducted.

The notifier occasionally has conducted in-vitro metabolism studies in cases when a special experiment could address specific questions related to the toxicological profile of an active substance to supplement the existing data or in an attempt to address the new data requirements of the regulation (EC) No. 1107/2009 after their publication.

However, a general call-in of comparative in-vitro metabolism studies to routinely address the non-specific requirements of the regulation (EC) No. 1107/2009 is deemed premature. Several methods to perform in-vitro assays are published in the literature. A variety of test parameters leads to numerous test parameter combinations and therefore to numerous possible tests. The objectives of the published experiments and the interpretation of the results vary in many aspects. In the absence of an adopted guideline, the appropriate selection of the study parameters is uncertain. The same applies to the evaluation and interpretation of the results. Therefore, no study was conducted for fluoxastrobin.

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

Not applicable, the toxicokinetic studies have been fully summarised and described under point 5.1.1

CA 5.2 Acute toxicity

Summary acute toxicity

Acute toxicity studies summarized in Table 5.2-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008), new studies are added.

Table 5.2-1 Summary of acute toxicity studies

Study type / Species	Sex	Results	Test substance Purity % a.s. (E:Z isomer ratio for a.s.)	Reference
Oral, fasted Rat	M/F	LD_{50}: >2000 mg/kg bw	HEC 5725, 98.9 (100:0)	[redacted]; 1996; M-012717-01-1
Oral, fasted Rat	M/F	LD_{50}: >2000 mg/kg bw	HEC 5725 N, 99.3 (92:8)	[redacted]; 1998; M-012735-01-1
Dermal, Rat	M/F	LD_{50}: >2000 mg/kg bw	HEC 5725, 100.2 (100:0)	[redacted]; 1998; M-012730-01-1
Inhalation, 1x4 h Rat	M/F	LC_{50}: >5 mg/L	Fluoxastrobin, 94.5 (99:1)	[redacted]; 1999; M-008820-01-1
Skin irritation Rabbit	M	not irritating	HEC 5725, 98.9 (100:0)	[redacted]; 1999; M-012662-02-1
Eye irritation Rabbit	M	slight reversible irritation	HEC 5725, 98.9 (100:0)	[redacted]; 1999; M-012669-02-1
Sensitization (Magnusson & Kligman) Guinea Pig	F	not sensitizing	HEC 5725, 98.9 (100:0)	[redacted]; 1996; M-012720-01-1 (BCS response M-070785-01-1)
Sensitization (Magnusson & Kligman) Guinea Pig	M	not sensitizing	HEC 5725, 99.6	[redacted]; 2003; M-105571-01-1
Sensitization (Magnusson & Kligman) Guinea Pig	F	not sensitizing	HEC 5725, 95.3	[redacted]; 2006; M-278315-01-1
Phototoxicity (BALB/c 3T3 cells)	-	not phototoxic	HEC 5725, 96.4	[redacted]; 2014; M-497574-01-1

Purity of a.s as stated in study report.
E:Z ratios from additional information supplied by applicant ([redacted]; 2002; M-077209-01-1)

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on acute toxicity:

The oral toxicity of fluoxastrobin is low, i.e. LD_{50} > 2000 mg/kg bw as well as inhalation LC_{50} >5 mg/l air. The toxicity via dermal route is low (LD_{50} >2000 mg/kg bw). It is not a skin or an eye irritant. No skin sensitisation potential was observed in a study with HEC 5725 (100%*E*). However, the material tested was of much higher purity than the preliminary proposed technical specification. The rapporteur Member State required the evaluation of the toxicological significance of impurities in fluoxastrobin in skin sensitisation. The issue was dealt with in the addendum 1 and it was concluded that only one impurity (C1) is specified at 1% or above (cut off criteria). Therefore a skin sensitisation study was performed with a batch containing 3.5 % of impurities of the technical specification for which approval is sought and a negative result was obtained, showing that fluoxastrobin impurities have no sensitising potential. The experts agreed with this conclusion.

After improvement of the production process (for details see document JCA 1.8), an additional sensitization study was conducted in order to support the new technical specification of fluoxastrobin. This study confirmed the absence of a skin sensitisation potential. Furthermore, fluoxastrobin does not show a phototoxic potential.

CA 5.2.1 Oral

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.2.2 Dermal

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.2.3 Inhalation

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.2.4 Skin irritation

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.2.5 Eye irritation

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.2.6 Skin sensitization

In addition to the studies on skin sensitization already available in the DAR and baseline dossier, a new Magnusson Kligman test was performed in 2006 with a representative final full production batch and submitted in order to support the new technical specification.

Report: KCA 5.2.6/04 [redacted], 2006, M-278315-01-1
Title: Fluoxastrobin (HEC 5725) (Project: Fluoxastrobin (HEC 5725)) - Study for the skin sensitization effect in guinea pigs (guinea pig maximization test according to Magnusson and Kligman)
Report No.: AT03336
Document No.: M-278315-01-1
Guideline(s): OECD 406; Guideline 96/54/EC, Method B.6.; US-EPA 712-C-03-197, OPPTS 870.2600
Guideline deviation(s): Analytical determinations of the stability of the paste in Cremophor EL/sterile physiological saline solution 2% v/v for administration were not performed.
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test material: Fluoxastrobin technical
Synonym(s): HEC 5725
Chemical name: (E)-Methanone, [2-[[6-(2-chlorophenoxy)-5-fluoro-4-pyrimidinyl]oxy]phenyl](5,6-dihydro-1,4,2-dioxazin-3-yl)-, O-methyloxime

Description:	White powder
CAS No.:	361377-29-9
Lot/Batch no:	BID 4012-143
Purity:	95.3% w/w
Stability of test compound:	Guaranteed for study duration; expiry date: 2007-06-2 Stability and homogeneity of test item formulations in the vehicle (1%-50%) analytically verified for up to 2 hours.)
2. Vehicle:	Cremophor EL/sterile physiological saline solution 2% v/v
3. Test animals	
Species:	Guinea pig
Strain:	Crl:HA
Sex:	Female
Age:	5-6 weeks
Weight at dosing:	342-403 g
Source:	[redacted], Germany.
Acclimatisation period:	At least 5 days
Diet:	PROVIMI KLIBA 3420 - Maintenance Diet for Guinea Pigs (PROVOMI KLIBA A), *ad libitum*
Water:	Tap water, *ad libitum*
Housing:	Noryl cages ([redacted], Germany) in groups of eight during the adaptation period and in groups of two or five per cage throughout the study period on Lignocel BK 8-15 low-dust wood shavings ([redacted], Germany)

B. Study design and methods

1. Animal assignment and treatment

Dose	
Intradermal induction:	5% (20 mg test item/animal)
Topical induction:	62.5% (500 mg test item/animal)
Challenge:	62.5% (500 mg test item/animal)
Application route:	Dermal
Application volume:	0.5 mL/patch
Exposure:	Topical induction: 48 hrs, challenge: 24 hrs
Group size:	35 animals (control: 10, test item: 20, range-finding: 5)
Observations:	Mortality, clinical signs, skin effects, body weight (at beginning and termination of study)

II. Results and discussion

A. Mortality and observations

One animal (no. 30) of the test item group died at day 12 of the study. Appearance and behaviour of the test item group were not different from the control group. At the end of the study, the mean body weight of the treatment group animals was in the same range than that of the control group animals.

After the intradermal induction (first induction) the animals in the control group and test item group showed red wheals at the injection sites after 48 hours. After 7 days at the injection sites encrustations were observed in the control group and wheals and encrustations were observed in the test item group.

B. Findings

The incidence of skin reactions following the challenge is summarized below:

Table 5.2.6-1: Number of animals exhibiting skin effects

	Test item group (19 animals)					Control group (10 animals)				
	Test item patch			Control patch		Test item patch			Control patch	
Hours	48	72	Total	48	72	48	72	Total	48	72
Challenge 62.5%	0	0	0	0	0	0	0	0	0	0

III. Conclusion

Under the conditions of the maximization test and with respect to the evaluation criteria fluoxastrobin exhibits no skin-sensitisation potential.

CA 5.2.7 Phototoxicity

According to the new data requirements (COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013, (1), the conduct of an in vitro phototoxicity study is required "*where the active substance absorbs electromagnetic radiation in the range 290-700 nm and is liable to reach the eyes or light-exposed areas of skin, either by direct contact or through systemic distribution. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance is less than 10 L x mol^{-1} x cm^{-1}, no toxicity testing is required.*"

Since this coefficient exceeds the trigger of 10 L x mol^{-1} x cm^{-1} for fluoxastrobin, a phototoxicity study was conducted.

Report: KCA 5.2.7/01 [redacted] 2014; M-497574-01-1
Title: Fluoxastrobin TC: Cytotoxicity assay in vitro with BALB/c 3T3 cells: Neutral red (NR) test during simultaneous irradiation with artificial sunlight
Report No.: 1611000
Document No.: M-497574-01-1
Guideline(s): Commission Regulation (EC) No 440/2008 B 41; Committee for Proprietary Medicinal Products (CPMP) Note for Guidance on Photosafety testing, EMEA, CPMP/SWP/398/01; OECD 432
Guideline deviation(s): none
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test materials:

Name:	Fluoxastrobin
Synonyms:	AE 1228646, technical substance
Description:	White powder
Lot/Batch no.:	HEG 21596-1-3
Purity:	96.4% (w/w) (dose calculation was not adjusted to purity)
Stability of test compound:	Guaranteed for study duration; expiry date: 2016-06-16

2. Vehicle and or positive control:

Vehicle:	Dimethylsulfoxide (DMSO), 1% (v/v) in Earle´s Balanced Salt Solution (EBBS)
Solvent control	EBSS containing 1% (v/v) DMSO
Positive control	Chlorpromazine dissolved in EBSS

3. Test system:

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

B. Study design and methods

1. Treatment:

Dose:

Test item	+/– UV	Final concentrations in µg/mL
Fluoxastrobin	+/–	0.49 0.98 1.95 3.91 7.81 15.6 31.3 62.5 (range finding (RFE))
		0.49 0.98 1.95 3.91 7.81 15.6 31.3 62.5 (main experiment (ME))
		0.24 0.49 0.98 1.95 3.91 7.81 15.6 31.3 (confirming experiment (CE))
Positive control	–	6.25, 12.5, 25, 37.5, 50, 75, 100, 200
	+	0.125, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0

Due to the limited solubility of the test item, the highest applied concentration of the test item in the RFE and in the ME was 62.5 µg/mL. The limit of solubility for the test item was obviously reached with this concentration, since the solution became turbid 5 minutes after preparation; a CE was performed with a reduced highest concentration of 31.3 µg/mL.

Solar simulator:	UVB irradiation kept as low as possible. The produced wavelength of the solar simulator with the filter was >320 nm. Due to the heterogeneous distribution of irradiation intensity the UVA intensity was measured at the complete area with a UV-meter. The homogeneous area was marked and the cultures were irradiated in this area. The solar simulator was switched on about 30 min prior to the start of experiment. The absorption spectrum of the test item was determined in the range from 270-800 nm. The test item showed absorption maxima at 272.9 and 278.0 nm.
Seeding of cultures:	2 x 104 cells per well were seeded in 100 µL culture medium in two 96-well plates (two plates, one was exposed to artificial sunlight, one was kept in the dark)
Replicates:	2 (one for exposure to irradiation, one for treatment in the dark, further replicates as described under “Treatment”. Solvent controls were measured 12 times)
Treatment & irradiation:	24 h after seeding the cultures were washed with EBSS. 100 µL of the dissolved test item were added/well and the plates were pre-incubated for 1 hour in the dark. Afterwards one plate was irradiated at 1.65 mW/cm2 (4.95 J/cm2) for 50 min at 25-28°C, the other plate was stored for 50 min at 25-28°C in the dark. The test item was removed and both plates were washed twice with EBSS. Fresh culture medium was added and the plates were incubated overnight at 37 ± 1.5 °C and 7.5% ± 0.5 CO2.

Cytotoxicity determination: For measurement of Neutral Red uptake the medium was removed and 0.1 mL serum-free medium containing 50 µg Neutral Red / mL were added to each well. The plates were incubated for another 3 hours at 37°C, before the medium was removed completely and the cells were washed with EBSS. For extraction of the dye 0.15 mL of a solution of 49% (v/v) deionized water, 50% (v/v) ethanol and 1% (v/v) acetic acid were added to each well. After approximately 10 min at room temperature and a brief agitation, the plates were transferred to a microplate equipped with a 540 nm filter to determine the absorbance of the extracted dye. This absorbance showed a linear relationship with the number of surviving cells.

2. Evaluation

The mean absorption (OD540) value per concentration was calculated. The ED50 values were determined by curve fitting by software. The Photo-irritancy factor (PIF), as well as the Mean Phototoxic effect (MPE) was calculated according to OECD guideline 432.

Evaluation criteria:
PIF < 2 or MPE < 0.1 => no phototoxic potential
PIF > 2 and < 5 or
MPE > 0.1 and < 0.15 => probable phototoxic potential
PIF > 5 or MPE > 0.15 => phototoxic potential

Acceptability criteria:
- after irradiation with a UVA dose of 5 J/cm² the cell viability of solvent control is >80% of non-irradiated cells
- the positive control PIF between the two ED50 values is >6
- the mean OD540 of solvent controls is >0.4

II. Results and discussion

In the range finding experiment (RFE) and in the confirming experiment (CE) cytotoxic effects did not occur after exposure of fluoxastrobin to the cells, neither in the presence nor in the absence of irradiation with artificial sunlight. Therefore, ED_{50}-values or a PIF could not be calculated. The resulting MPE values were 0.053 and 0.009, respectively.

In the main experiment (ME) a slight cytotoxic effect occurred after irradiation of the highest tested concentration of 62.5 µg/mL. The cell viability decreased to 60.40%. In the non-irradiated test group cytotoxicity was not detected. Since the viability was not reduced below 50%, ED_{50} values could not be calculated, following also a PIF value could not be determined. The MPE value was calculated as 0.154 indicating a phototoxic potential.

However, the reason for the differing results of the RFE and the ME is most likely slight turbidity observed 5 minutes after preparation of the highest test item concentration of 62.5 µg/mL of the RFE and of the ME solutions. Obviously, the limit of solubility of the test item was reached with the concentration of 62.5 µg/mL. According to the OECD guideline no test item precipitation should occur in the irradiated cultures. Therefore, the confirmatory experiment was performed with a reduced highest test item concentration of 31.3 µg/mL which completely confirmed the results of the RFE. According to these results the test item is classified as not phototoxic.

The mean of solvent control values of the irradiated versus the non-irradiated group met the acceptance criteria. The positive control chlorpromazine induced phototoxicity in the expected range in the presence of irradiation.

The results are summarised in the following tables.

Table 5.2.7-1: Optical density at 540 nm (OD_{540} values) in the Neutral Red assay of the range finding experiment (RFE)

	OD_{540} with artificial sunlight				OD_{540} without artificial sunlight		
Con-centration [µg/mL]	**Mean**	**SD**	**% of solvent control**	**Con-centration [µg/mL]**	**Mean**	**SD**	**% of solvent control**
Treatment with fluoxastrobin							
Solvent control	0.6932*	0.0167	100.00	**Solvent control**	0.7039*	0.0285	100.00
0.49	0.7348	0.0247	105.99	**0.49**	0.7477	0.0151	106.23
0.98	0.7025	0.023	101.34	**0.98**	0.7659	0.0304	108.8
1.95	0.6944	0.0403	100.16	**1.95**	0.7576	0.0168	107.62
3.91	0.6834	0.0404	98.58	**3.91**	0.7205	0.0098	102.35
7.81	0.6684	0.0527	96.41	**7.81**	0.733	0.0204	103.71
15.6	0.6277	0.0294	90.55	**15.6**	0.7235	0.0236	102.78
31.3	0.6414	0.0126	92.52	**31.3**	0.6948	0.0101	98.71
62.5	0.5875	0.0376	84.75	**62.5**	0.5894	0.0253	83.73
Treatment with positive control chlorpromazine							
Solvent Control	0.7168*	0.086	100	**Solvent Control**	0.7314*	0.0226	100
0.125	0.7001	0.0462	97.67	**6.25**	0.728	0.0226	99.52
0.250	0.0693	0.0058	9.67	**12.50**	0.467	0.0281	63.84
0.500	0.0712	0.0031	9.94	**25.00**	0.1789	0.0359	24.46
0.750	0.073	0.0065	10.18	**37.50**	0.0678	0.0057	9.27
1.000	0.0701	0.0049	9.78	**50.00**	0.0751	0.0063	10.26
1.500	0.0677	0.0053	9.45	**75.00**	0.0569	0.005	7.77
2.000	0.0682	0.0048	9.51	**100.00**	0.0564	0.0045	7.72
4.000	0.0727	0.0086	10.15	**200.00**	0.0584	0.0046	7.98

* mean OD_{540} out of 12 wells

Table 5.2.7-2: Optical density at 540 nm (OD_{540} values) in the Neutral Red assay of the main experiment (ME)

	OD_{540} with artificial sunlight				OD_{540} without artificial sunlight		
Con-centration [µg/mL]	**Mean**	**SD**	**% of solvent control**	**Con-centration [µg/mL]**	**Mean**	**SD**	**% of solvent control**
Treatment with fluoxastrobin							
Solvent control	0.8069*	0.0822	100	**Solvent control**	0.7375*	0.1466	100
0.49	0.8381	0.0832	103.86	**0.49**	0.7922	0.0839	107.41
0.98	0.8198	0.0489	101.6	**0.98**	0.7848	0.0343	106.42
1.95	0.7513	0.0855	93.11	**1.95**	0.7508	0.0185	101.8
3.91	0.7187	0.058	89.07	**3.91**	0.7591	0.0513	102.93
7.81	0.688	0.0608	85.26	**7.81**	0.7649	0.0271	103.71
15.6	0.6607	0.0705	81.88	**15.6**	0.754	0.0175	102.24
31.3	0.5694	0.059	70.57	**31.3**	0.7289	0.0339	98.84
62.5	0.4874	0.0576	60.4	**62.5**	0.6223	0.0537	84.38
Treatment with positive control chlorpromazine							
Solvent Control	0.7433*	0.043	100	**Solvent Control**	0.7407*	0.0694	100
0.125	0.669	0.0661	90.01	**6.25**	0.7476	0.0727	100.92
0.250	0.1044	0.0676	14.05	**12.50**	0.5106	0.0435	68.94
0.500	0.0683	0.0053	9.18	**25.00**	0.1071	0.0183	14.46
0.750	0.0706	0.0067	9.5	**37.50**	0.0741	0.0079	10
1.000	0.0744	0.0074	10.01	**50.00**	0.0666	0.0074	8.99
1.500	0.1002	0.0595	13.48	**75.00**	0.0678	0.0105	9.16
2.000	0.0719	0.0052	9.68	**100.00**	0.0755	0.0217	10.19
4.000	0.0721	0.0055	9.69	**200.00**	0.081	0.0475	10.93

*: mean OD_{540} out of 12 wells

Table 5.2.7-3: Optical density at 540 nm (OD_{540} values) in the Neutral Red assay of the confirming experiment (CE)

	OD_{540} with artificial sunlight				OD_{540} without artificial sunlight		
Con-centration [µg/mL]	**Mean**	**SD**	**% of solvent control**	**Con-centration [µg/mL]**	**Mean**	**SD**	**% of solvent control**
Treatment with fluoxastrobin							
Solvent Control	0.6533*	0.087	100	**Solvent Control**	0.7051*	0.0515	100
0.24	0.6918	0.1229	105.9	**0.24**	0.7288	0.1039	103.35
0.49	0.6795	0.0698	104.02	**0.49**	0.6862	0.0271	97.31
0.98	0.6306	0.0417	96.52	**0.98**	0.706	0.0248	100.11
1.95	0.6136	0.029	93.92	**1.95**	0.6925	0.0388	98.21
3.91	0.6287	0.0227	96.24	**3.91**	0.7725	0.0771	109.55
7.81	0.6364	0.0459	97.41	**7.81**	0.6965	0.0442	98.77
15.6	0.6516	0.0319	99.74	**15.6**	0.6951	0.0137	98.57
31.3	0.625	0.0278	95.67	**31.3**	0.6699	0.0164	95
Treatment with positive control chlorpromazine							
Solvent Control	0.7103*	0.0873	100	**Solvent Control**	0.6915*	0.1104	100
0.125	0.632	0.0295	88.98	**6.25**	0.5997	0.0278	86.72
0.250	0.2055	0.074	28.93	**12.50**	0.2007	0.0719	29.02
0.500	0.0628	0.0097	8.84	**25.00**	0.0624	0.0088	9.02
0.750	0.0712	0.0276	10.02	**37.50**	0.0711	0.0262	10.28
1.000	0.0573	0.0057	8.07	**50.00**	0.057	0.0057	8.24
1.500	0.0543	0.0018	7.65	**75.00**	0.054	0.0018	7.8
2.000	0.0571	0.002	8.04	**100.00**	0.0568	0.0021	8.22
4.000	0.055	0.0025	7.74	**200.00**	0.0544	0.0025	7.86

*: mean OD_{540} out of 12 wells

Table 5.2.7-4: Summary of the results of the Neutral Red assay

	Substance	**ED_{50} (+UV) [µg/mL]**	**ED_{50} (-UV) [µg/mL]**	**PIF**	**MPE**	**% viability of solvent control of irradiated *vs.* non-irradiated plate**
Range finding experiment	Fluoxastrobin	--	--	--	0.053	98.5
	Positive control	0.18	14.83	83.63	0.787	98.0
Main experiment	Fluoxastrobin	--	--	--	0.154	109.4
	Positive control	0.18	14.53	80.84	0.739	100.3
Confirming experiment	Fluoxastrobin	--	--	--	0.009	92.6
	Positive control	0.20	9.57	48.36	0.706	102.7

PIF: Photo-Irritancy-Factor MPE: Mean Phototoxic Effect
--: No cytotoxic effects occurred after exposure of to the cells, neither with nor without irradiation with artificial sunlight. Therefore, ED_{50}-values or a PIF could not be calculated.

III. Conclusion

Based on the study results fluoxastrobin does not possess any phototoxic potential.

CA 5.3 Short-term toxicity

Summary short-term toxicity

Short-term toxicity studies summarized in Table 5.3-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008).

Table 5.3-1 Summary of short-term toxicity studies

Study Doses tested	Sex	NO(A)EL ppm (mg/kg bw/day)	LO(A)EL ppm (mg/kg bw/day)	Main findings at LO(A)EL	Reference
Rat					
28-day oral (diet) 0-100-500-2500-10000 ppm (M/F: 0-12/11-64/55-383/265-1930/1441 mg/kg bw/d) (100% *E*)	M F	100 (12) 100 (11)	500 (64) 500 (55)	Adrenal pathology (small cytoplasmic vacuoles) Reduced hepatic activity (plasma triglycerides ↓)	[redacted]; 1997; M-012683-01-1
28-day oral (diet) 0-100-500-2500-10000 ppm (M/F: 0-10/9-50/43-237/222-1017/892 mg/kg bw/d) (92:8 *E:Z*)	M F	100 (10) 500 (43)	500 (50) 2500 (222)	Reduced hepatic activity (N-demethylase activity ↓) Reduced body weight gain	[redacted]; 1999; M-017457-01-1
28-day oral (diet) 0-100-500-2500-10000 ppm comparison of Fluoxastrobin (99:1 *E:Z*) (M/F: 0-8/10-42/53-210/261-1006/1452 mg/kg bw/d) HEC 5725 A (63:35 *E:Z*) (M/F: 0-7/7-34/38-181/198-801/1136 mg/kg bw/d)	M F	100 (7-8) 500 (38-53)	500 (34-42) 2500 (198-261)	Reduced hepatic activity (N-demethylase activity↓) Hepatic effects (AST ↓*, ALT ↓, N-demethylase activity ↓); adrenal pathology (cytomegaly in the cortex) <u>Other comment:</u> Both materials show very similar NOAEL and LOAEL (mg/kg bw/d) and the same spectrum of effects	[redacted]; 2002; M-040721-01-1
90-day oral (diet) 0-125/250-1000/2000-8000/16000 ppm (M/F: 0-9/22-70/163-580/1416 mg/kg bw/d) (100% *E*)	M F	125 (9) 2000 (163*)	1000 (70) 16000 (1416)	Reduced hepatic function (plasma triglycerides ↓) Reduced hepatic function (plasma triglycerides ↓), urinary tract lesions (one animal), *serum ALT ↓ ~~borderline of~~ not an adverse effect	[redacted]; 1998; M-012710-01-1
4-week dermal 0-100-300-1000 mg/kg bw (99:1 *E:Z*)	M F	1000 1000	>1000 >1000	No adverse effects seen at the top dose of 1000 mg/kg bw	[redacted]; 2000; M-027714-01-1

Study Doses tested	Sex	NO(A)EL ppm (mg/kg bw/day)	LO(A)EL ppm (mg/kg bw/day)	Main findings at LO(A)EL	Reference
Mouse					
2-week oral (diet) 0-100-450-1800 ppm (M/F: 0-20/37-92/115-354/571 mg/kg bw/d) (99:1 *E:Z*)	M	450 (92)	1800 (354)	Reduced hepatic activity (reduced serum ALT)*	[redacted]; 1999; M-018299-02-1
	F	450 (115)	1800 (571)	non-GLP limited endpoints	
3-month oral (diet) dose-ranging study 0-450-1800-7000 ppm (M/F: 0-81/135-313/539-1304/2257mg/kg bw/d) (100% *E*)	M	< 450 (< 81)	450 (81)	Reduced hepatic activity (reduced serum ALT primarily in females)*	[redacted]; 1998; M-012706-01-1
	F	< 450 (< 135)	450 (135)		
Dog					
90-day oral (diet) 0-25-50 ppm (M/F: 0-0.7/0.7-1.4/1.5 mg/kg bw/d) (99:1 *E:Z*)	M	50 (1.4**)	> 50 (> 1.5)	No adverse effects seen	[redacted]; 2001; M-088674-01-1
	F	50 1.5	> 50 1.5		
90-day oral (diet) 0-100-800-2500 ppm (M/F: 0-3/3-25/24-76/75 mg/kg bw/d) (99:1 *E:Z*)	M	< 100 (3**)	100 (25)	Reduced bw gain	[redacted]; 2001; M-088684-02-1
	F	100 (3**)	800 (24)	Reduced bw gain and hepatic function (PROT ↓, ALB ↓), increased serum alkaline phosphatase	
1-year oral (diet) 0-25-50-250-1200 ppm (M/F: 0-0.8/0.7-1.7/1.6-8/8-35/37 mg/kg bw/d) (99:1 *E:Z*)	M	50 (1.7)	250 (8)	Increased serum alkaline phosphatase	[redacted]; 2002; M-088509-02-1 BCS response: M-057922-01-1
	F	50 (1.5)	250 (8)	Reduced bw gain and increased serum alkaline phosphatase	

*serum ALT/AST reduction ~~borderline~~ not an adverse effect (please refer to DAR Addendum 1), ** for setting overall 90-day NOAEL in dogs taking into account effects seen in 1-year study), body weight effects are not considered to be the critical effect.

Details can be taken from the DAR and Addendum 1 to the DAR:

Dietary studies in rats

Males were more sensitive than females to the effects of fluoxastrobin/HEC 5725 on the liver and urinary tract.

Changes in enzyme activity in liver tissue were seen: reductions in cytochrome P-450 related enzymes and increases in some phase II enzymes. Reductions in serum levels of triglycerides, ALT, AST, alkaline phosphatase also provide evidence of reductions in certain hepatic activity. Hepatic effects in the 90-day rat study were reversible. Limited histopathological evidence of hepatic changes was seen only in 28-day studies: hepatocytomegaly with HEC 5725 A (63%:35% *E:Z*, mostly at a high dose) and reduced glycogen at a high dose of HEC 5725 (100% E). Reduced liver cell proliferation was observed at a high dose in a 28-day study with HEC 5725 (100% E).

Kidney/urethra/bladder lesions (calculi and /or hyperplasia/inflammation), increased calcium oxalate crystals in urine and increased serum calcium levels were seen at high doses in the 90-day rat study.

These changes were further investigated in a special 9-week dietary study in rats, (please refer to MCA 5.8.2).

Two types of adrenal lesion showed an increased incidence: uniformly small cytoplasmic vacuoles in two studies (shown to be reversible), adrenal cytomegaly in another study at the top dose.

Some slight reductions in red blood cell parameters were seen at a high dose in the 90-day study.

No substance-related adverse immunotoxic effects were seen, which agrees with the finding of a special immunotoxicity study in mice and a new immunotoxicity study in rats (please refer to MCA 5.8.2).

Fluoxastrobin (99% E: 1%E) and HEC 5725 A (63% E: 35%Z) were shown to have an almost identical toxicity profile and NOAEL in a 28-day study. This response was also generally similar to the findings from 28-day studies with HEC 5725 (100% E) and HEC 5725 N (92% E: 8%Z).

Dietary studies in mice

Studies in mice indicated they were not a particularly sensitive species to the effects of fluoxastrobin.

In the range-finding 90-day study with HEC 5725 (100% E), histopathological changes in the liver (indicative of induction and toxicity) and reduced red blood cell parameters were reported at high dose levels.

In a subsequent special 2-week study with fluoxastrobin at doses of up to 3540.571 mg/kg bw/day, there was evidence for hepatic induction (increased glutathione S-transferase) and for reduced hepatic activity (reduced serum alanine aminotransferase activity). Liver cell proliferation was increased but this is not considered to be a clear adverse effect in the absence of proliferative hepatic lesions in the 90-day and carcinogenicity studies with mice.

Dietary studies in dogs

Reduced body weight gain was a key finding in dog studies with fluoxastrobin. However the findings were not consistent between studies with respect to the effect on body weight gain over the first 3 months (see Table B.6.20 of the DAR). Taking into account the observed variability, it is considered that 250 ppm (8 mg/kg bw/day) was the overall NOAEL for effects on body weight after exposure for 3 months (from days 0 to 84 or 91) based on effects at 800 ppm and above. (The overall 90-day NOAEL for dogs is based on increased serum alkaline phosphatase, see below).

Reduced body weight gain was also seen over the first week of exposure at 800 ppm and above and this seemed to be due in large measure to a reduction in food consumption over this period, (see Table B.6.20 of the DAR). However as mean body weight loss of 0.5-0.7 kg was seen over the first week at 3,000 ppm it would seem prudent to consider 1,200 ppm as a NOAEL of possible relevance for setting an acute reference dose. Mean test substance intakes over the first week of exposure were c. 40 mg/kg bw/day at 3,000 ppm and c. 30 mg/kg bw/day at 1,200 ppm.

There was evidence of both hepatic induction (eg hepatocytomegaly, increased cytochrome P450 activity) and impaired function activity (eg reduced serum albumin and cholesterol and increased alanine aminotransferase and gamma glutamyl transpeptidase). Marked increases in absolute and relative liver weights of females also suggest potentially adverse effects but there was no histopathological evidence of liver damage.

Hepatic induction probably accounted for the observed transient decrease in serum levels of the thyroid hormone T4 in a 90-day study, which is consistent with the increase in UDP-glucuronosyltransferase activity. The study investigators also propose that hepatic induction may have resulted in reduced erythrocyte viability in a 90-day study.

Kidney effects included degenerative histological changes and increased pigmentation.

Alkaline phosphatase activity was increased in dogs (it is notable that the serum activity of this enzyme was reduced in the 90-day rat study). Although it is possible that this increased enzyme

activity could reflect hepatic induction, it can also be indicative of toxicity to liver, intestine and bone. It is considered necessary to regard the increased activity as a potentially adverse effect because:

- there was evidence of impaired hepatic function in the dog (although increases in alkaline phosphatase were seen at a lower dose than clear increases in alanine aminotransferase and gamma glutamyl transferase).
- the potential for effects on bone (effects were seen in rats at the top dose after 2 years, see Section B.6.8.4.c of the DAR). It is notable that reduced serum calcium levels were observed in dogs, and bone was investigated directly in dogs only by histopathological examination following standard H and E staining (there were no specialised investigations of bone in dogs).

Dermal study in rats

In a 4-week dermal study with fluoxastrobin in rats, neither systemic nor local skin effects of toxicological importance were observed up to the highest dose level tested (1,000 mg/kg bw/day). The study included an investigation of some clinical chemistry parameters of hepatic activity but there was no investigation of effects on calcium and phosphorus homeostasis. Fluoxastrobin was moistened with water, which is not representative of the EC formulation for which approval is sought.

Overall 90-day NOAEL in dogs is 100 ppm (3 mg/kg bw/day) based on increased serum alkaline phosphatase at 250 ppm after 87 days in the 1-year dog study. This is also supported by effects at 800 ppm in the first 90-day dog study.

Conclusion from the EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on short-term toxicity:

The short-term toxicity of fluoxastrobin has been investigated in dietary studies in rats (28-day and 90-day studies), mice (2-week and 90-day studies) and dogs (90-day and 1-year studies). A 28-day dermal toxicity study in rats has also been conducted.

The liver is the main target organ in all tested species (rats, mice and dogs). Histological changes were seen in the urinary system of rats (high doses) and dogs. Male rats were more sensitive than females to the effects of fluoxastrobin/HEC 5725 on the liver and urinary tract. Other target organs were adrenals, erythrocytes and thyroid. Reduced body weight gain was a key finding in dog studies.

In a 28-day dermal study with fluoxastrobin in rats, neither systemic nor local skin effects of toxicological importance were observed up to the highest dose level tested (1000 mg/kg bw/day).

No repeated dose inhalation were submitted, nor required.

The NOAEL in the 1-year dog study is 1.5 mg/kg bw/day (time point 12 months). The overall short term NOAEL in dogs is 3 mg/kg bw/day based on increased serum alkaline phosphatase at 8 mg in the 1-year dog study at the 90-day time point. This is also supported by effects observed at 24 mg/kg bw/day in the first 90-day dog study.

CA 5.3.1 Oral 28-day study

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.3.2 Oral 90-day study

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.3.3 Other routes

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.4 Genotoxicity testing

Summary genotoxicity

Genotoxicity tests summarized in Table 5.4-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008), new studies are added.

Table 5.4-1: Summary of genotoxicity studies

Test System	Concentration/ Dose	Results	Test item, % purity (E: Z isomer ratio)	Reference
In vitro				
Ames test	Up to 5000 µg/plate (plate incorporation) Up to 3162 µg/plate (with pre-incubation)	Negative	HEC 5725, 98.9 (100:0)	[redacted]; 1996; M-012700-01-1
Ames test	Up 5000 µg/plate (plate incorporation and pre-incubation)	Negative	HEC 5725 N, 99.7 (99:1)	[redacted]; 1998; M-012732-01-1
Ames test	Up 5000 µg/plate (plate incorporation and pre-incubation)	Negative	Fluoxastrobin 95.3	[redacted]; 2006; M-278050-01-1
Chromosome aberration V79 cells	Up to 320 µg/ml	Negative	HEC 5725, 98.9 (100:0)	[redacted]; 1996; M-012703-01-1
Forward mutation assay V79-HPRT	Up to 200 µg/ml	Negative	HEC 5725, 98.9 - 99.4 (100:0)	[redacted]; 1997; M-012722-01-1
Forward mutation assay V79-HPRT	Up to 160 µg/ml	Negative *	Fluoxastrobin 94.7 (99:1)	[redacted]; 2003; M-078586-01-1
In vivo				
Micronucleus test NMRI mice	Up to 300 mg/kg bw/day, administered on 2 days b.i.p	Negative Clear evidence of systemic toxicity and/or fluoxastrobin and/or its metabolites reaching the bone marrow	Fluoxastrobin, 94.5 (99:1)	[redacted]R; 1999; M-012747-01-1

* Some uncertainty as to the sensitivity of this type of assay. RMS prefers a mouse lymphoma assay.

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on genotoxicity:

There is no evidence of genotoxic potential of fluoxastrobin in any of the submitted genotoxicity studies. However, most of *in vitro* studies were conducted on material of higher purity than that for which approval is sought.

Hence for additional reassurance that the impurities in fluoxastrobin are not of genotoxic concern, the applicant was asked to conduct an Ames study with a representative final full production batch. The issue of toxicological effects of impurities has been discussed in the experts' meeting where a review of the toxicity data on different batches and impurities summarised in the addendum 1 to the DAR was reviewed. The meeting agreed that a satisfactory investigation of the impurities had been performed and no further genotoxicity data were required.

A new Ames test was performed in 2006 with a representative final full production batch which also supports the new technical specification. The new Ames test confirmed that there is no evidence of a genotoxic potential of fluoxastrobin.

Photomutagenicity
According to the new data requirements (COMMISSION REGULATION (EU) No 283/2013 of 1 March 2013; Official Journal of the European Union, L 93/1, 3.4.2013), special testing requirements in relation to photomutagenicity may be indicated by the structure of a molecule. If the Ultraviolet/visible molar extinction/absorption coefficient of the active substance and its major metabolites is less than 1000 L × mol^{-1} × cm^{-1}, photomutagenicity testing is not required.

As described by [redacted] et al., 2011 (Considerations on photochemical genotoxicity. II: Report of the 2009, International Workshop on Genotoxicity Testing Working Group, M-528387-01-1), photogenotoxicity testing and photosafety testing in general should follow a tiered approach.

The first tier is the molar extinction coefficient, with no photosafety testing required for compounds with a molar extinction coefficient below 1000 L × mol^{-1} × cm^{-1}. As the molar extinction coefficient of fluoxastrobin exceeds this limit, it was tested in a standard phototoxicity study and was shown to be negative (see MCA 5.2.7, document M-497574-01-1).

The second step of the evaluation process is triggered by the results of the phototoxicity study, and the following is found on page 99 of the cited reference:
"If an in vitro 3T3 NRU phototoxicity test is negative there is no need for a photogenotoxicity study. Given the similarity of the underlying principles involved in inducing the different endpoints it is very unlikely that a clearly non-phototoxic compound could have a relevant photogenotoxic potential."
Based on this statement by the International Working Group on Genotoxicity Testing in 2009, photomutagenicity testing of fluoxastrobin is not triggered and is not required.

Moreover, for photomutagenicity testing agreed test methods or guidance documents are not yet available.

CA 5.4.1 In vitro studies

In addition to the genotoxicity tests already available in the DAR baseline dossier, a new Ames test was performed in 2006 with a representative final full production batch in order to support the new technical specification.

Report:	KCA 5.4.1/07 [redacted] R; 2006; M-278050-01-1
Title:	Fluoxastrobin (HEC 5725) (tested as Fluoxastrobin technical) - Salmonella/microsome test - Plate incorporation and preincubation method
Report No.:	AT03333
Document No.:	M-278050-01-1
Guideline(s):	OECD 471; Directive 2000/32/EC, Method B.13/14.; US-EPA 712-C-98-247, OPPTS 870.5100
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:	Fluoxastrobin technical
Description:	Fine white powder
Batch no:	BID 4012-143
Purity:	95.3%
Stability of test compound:	guaranteed for study duration; expiry date: 2007-06-26
2. Vehicle and/or positive control:	DMSO: Sodium azide (Na-azide), Nitrofurantoin (NF), 4-nitro-1,2-phenylene diamine (4-NPDA), Cumene hydroperoxide (Cumene), 2-aminoanthracene (2-AA) deionised water mitomycin C (MMC)
3. Test system:	*Salmonella typhimurium* strains TA1535, TA1537, TA100, TA98, TA102
Metabolic activation:	S9 mix

B. Study design and methods

Dose:	Fluoxastrobin: 0-16-50-158-500-1581-5000 µg/plate	
	Positive controls: Na-azide:	10 µg/plate
	NF:	0.2 µg/plate
	4-NPDA:	0.5+10 µg/plate
	MMC:	0.2 µg/plate
	Cumene:	50 µg/plate
	2-AA:	3 µg/plate
Application volume:	0.1 mL/plate	
Incubation time:	48 hrs, 37°C	

II. Results and discussion

Doses up to and including 5000 µg per plate fluoxastrobin, showed no bacteriotoxic effects. Substance precipitation started at 1581 µg per plate. Therefore, 5000 µg per plate could not be used for assessment.

Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective negative controls.

In spite of the low doses used, positive controls increased the mutant counts significantly compared with negative controls, and thus demonstrated the system's high sensitivity.

Despite this sensitivity, no indications of mutagenic effects of fluoxastrobin could be found at assessable doses of up to 1581 µg per plate in any of the *Salmonella typhimurium* strains used.

Table 5.4.1-1: Summary of results

Substance	Dose (µg/plate)	S9 mix	Mean revertants per plate: Strain TA1535	TA100	TA1537	TA98	TA102
Plate incorporation							
Fluoxastrobin	0	–	11	97	7	26	174
	16	–	13	107	5	26	200
	50	–	16	107	5	26	180
	158	–	13	105	6	28	187
	500	–	11	95	5	28	182
	1581	–	10	104	3	21	210
	5000	–	-	73	-	19	-
Na-azide	10	–	663				
NF	0.2	–		262			
4-NPDA	10/0.5	–			70	157	
MMC	0.2	–					474
Fluoxastrobin	0	+	14	110	9	38	237
	16	+	13	108	8	40	255
	50	+	12	123	8	45	286
	158	+	10	114	6	43	274
	500	+	9	126	6	39	235
	1581	+	9	118	6	41	194
	5000	+	-	-	-	-	-
2-AA	3	+	155	1290	255	1091	664
Pre-incubation							
Fluoxastrobin	0	–	12	136	6	19	213
	16	–	10	136	6	17	233
	50	–	12	139	8	19	231
	158	–	14	138	6	20	228
	500	–	14	151	6	14	242
	1581	–	16	144	4	13	202
	5000	–	-	107	-	-	-
Na-azide	10	–	662				
NF	0.2	–		493			
4-NPDA	10/0.5	–			116	141	
Cumene	550	–					445
Fluoxastrobin	0	+	14	185	10	29	299
	16	+	13	191	7	33	273
	50	+	13	190	7	24	257
	158	+	10	201	9	29	254
	500	+	10	166	10	30	274
	1581	+	12	194	7	31	272
	5000	+	-	-	-	-	-
2-AA	3	+	142	1487	335	1152	615

III. Conclusion

Fluoxastrobin has to be regarded as non-mutagenic.

CA 5.4.2 In vivo studies in somatic cells

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.4.3 In vivo studies in germ cells

Overall it is concluded that fluoxastrobin did not show a genotoxic potential and no evidence of an effect on germ cells was seen in other toxicological studies. Therefore, an *in vivo* study in germ cells is not regarded necessary.

CA 5.5 Long-term toxicity and carcinogenicity

Summary long-term toxicity

Long-term and carcinogenicity studies summarized in Table 5.5-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008).

Table 5.5-1 Summary of long-term and carcinogenicity studies

Study type Doses tested	Sex	NOAEL / LOAEL ppm (mg/kg bw/day)		Findings at LO(A)EL	Reference
2-year chronic tox. /carcinogenicity rat (diet) M: 0-40-100-1000-5000 ppm (0-2-5-53-272 mg/kg bw/day) F: 0-100-500-2500-12500 ppm (0-7-35-181-1083 mg/kg bw/day)	M	1000 (53)	5000 (272)	reduced bw gain	[redacted]; 2001; M-137193-01-1 BCS response M-057922-01-1, M-082214-01-1 BCS response update M-549514-01-1
	F	500 (35)	2500 (181)	reduced bw gain	
				test substance not oncogenic	
18-month carcinogenicity mouse (diet) 0-100-700-4200 ppm (M: 0-19-135-776 mg/kg bw/day F: 0-30-204-1265 mg/kg bw/day)	M	100 (135)	4200 (776)	incr. relative liver weight, (reduced plasma ALT)*	[redacted]; 2001; M-072442-01-1 BCS response M-057922-01-1
	F	100 (30)	700 (204)	(reduced plasma ALT)* No adverse effect observed at the top dose	
				test substance not oncogenic	

M: males, F: females; * not considered adverse (see DAR Addendum 1)

EFSA Scientific Report (2007) 102, 1-84: "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on long-term toxicity:

A chronic toxicity/ carcinogenicity study in rats and a carcinogenicity study in mice with fluoxastrobin (99% *E* : 1% *Z*) were conducted.

There was no evidence of a substance-related oncogenic response in either species. A higher incidence of uterine adenocarcinoma in high dose rats compared to concurrent controls was noted; possible influences of fluoxastrobin on the female endocrine system (including mechanistic information) was discussed at the experts' meeting. The applicant provided further information (particularly for controls in the concurrent study mentioned in the DAR, M-082214-01-1; as requested by RMS UK in their letter COP 2016/00206, Ref. W001721642, BCS response is updated in new document M-549514-01-1) to support the view that the increased incidence of uterine lesions at the top dose (adenocarcinoma and focal glandular hyperplasia) are not substance related and hence are not of concern for hazard or risk assessment of fluoxastrobin. Notably:

1. Occurrence of these tumours was similar in high dose and study controls, and also as compared with controls in a concurrent study.
2. The incidence of focal and diffuse glandular hyperplasia at the top dose was lower than the incidence of glandular cystic hyperplasia in controls in a concurrent study (the applicant indicates that, although the terminology differs slightly, the lesions are comparable).

3. As reported in the DAR, incidence of adenocarcinoma at the top dose was lower than in controls in the concurrent study.
4. There were no significant effects on reproductive performance in the multi-generation study with fluoxastrobin (indicating that fluoxastrobin does not induce endocrine effects).

In addition to glandular hyperplasia, also endometrial hyperplasia and metaplasia were seen during the study. The rapporteur Member State considered that these other hyperplastic lesions do not support the evidence of a substance related effect.

The experts' meeting agreed that the historical control data and particularly data from a study run concurrently suggested that the finding of uterine adenocarcinoma was incidental and that the concurrent control was low.

~~Adverse~~ Adaptive effects on the liver (reduced functional capacity, as shown by reduced plasma ALT and/or AST) were seen in both rats and mice. Increased liver weight and hepatocellular hypertrophy were also seen in mice.

There was evidence of altered calcium and phosphate homeostasis in rats, notably decreased phosphate excretion and decreased calcium content of bone. However there were no clear substance related pathological effects on the kidney or urinary bladder of rats or mice.

Caecal enlargement and increased number of mast cells in mesenteric lymph nodes were observed in high dose rats. The increase in mast cells was considered to be a local substance related effect and not to be indicative of an important substance-related immunological response.

CA 5.6 Reproductive toxicity

Summary reproductive toxicity

Reproductive and developmental toxicity studies summarized in Table 5.6-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008).

Table 5.6-1: Summary of reproductive and developmental toxicity studies

Study type	NOAEL	LOAEL	Effects at LOAEL	Reference
2-generation rat (diet) Dose: 0-100-1000-10000 ppm (Premating: M: 0-6.8-74-764 mg/kg bw/d F: 0-8.1-87-871 mg/kg bw/d Gestation: 0-7-75-742 mg/kg bw/d Lactation: 0-16-171-1625 mg/kg bw/d)	*Parental toxicity* 1000 ppm (74-87 mg/kg bw/d) *Reproductive outcome:* 10000 ppm (742-764 mg/kg bw/d) *Developmental toxicity* 1000 ppm* (1671 mg/kg bw/d in lactating dams)	*Parental toxicity* 10000 ppm (764-871 mg/kg bw/d) *Reproductive outcome:* >10000 ppm (>742-764 mg/kg bw/d) *Developmental toxicity* 10000 ppm* (1625 mg/kg bw/d in lactating dams)	Reduced bw gain, incr. liver weight, reduced thymus weight No adverse effects on reproductive outcome Reduced body weight gain, delayed development (delay in preputial separation) reduced thymus and spleen weight	[redacted],; [redacted]; 2004; M-088589-02-1
Developmental toxicity, rat (gavage) Dose: 0-100-300-1000 mg/kg bw/d	*Maternal toxicity* 1000 mg/kg bw/d *Developmental toxicity* 1000 mg/kg bw/d	*Maternal toxicity* >1000 mg/kg bw/d *Developmental toxicity* >1000 mg/kg bw/d	No adverse effects No adverse effects No teratogenic effects	[redacted],; [redacted]; [redacted]; 1997; M-012725-01-1
Developmental toxicity, rabbit (gavage) Dose: 0-25-100-400 mg/kg bw/d	*Maternal toxicity* 25 mg/kg bw/d *Developmental toxicity* 100 mg/kg bw/d	*Maternal toxicity* 100 mg/kg bw/d *Developmental toxicity* 400 mg/kg bw/d	Reduced food consumption, slight incr. incidence of distinct weight loss Slight dilation of brain ventricles No clear evidence of teratogenic effects	[redacted],; 1999; M-017448-01-1

~~* A conservative NOAEL based on only a slight effect (reduced thymus weight) in pups at 1,000 ppm.~~
Addendum 4 to the DAR (August 2004): The additional histological investigation of the thymus of F2 pups from control and 1000 ppm dose groups provided sufficient evidence to support raising the NOAEL for developmental effects in the rat multigeneration study to 1000 ppm, which is in line with the applicant´s proposal. At the next higher dose (10000 ppm) there were clear adverse effects on pups (reduced body weight gain, delayed development, reduced thymus and spleen weight).

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on reproductive and developmental toxicity:

A 2-generation reproductive toxicity study in rats and a developmental toxicity study in rabbits were conducted with a batch of fluoxastrobin that was quantitatively very similar to the preliminary proposed technical specification. The developmental toxicity study in rats was conducted with HEC

5725 (a.s 100% E isomer, 98.9% purity) which was of higher purity than the preliminary proposed technical specification.

In the 2-generation study, adverse developmental effects, ie reduced body weight gain, delayed development (e.g. time to preputial separation) and reduced weight of thymus and spleen of pups were seen at the top dose. NOAEL for reproduction is 10000 ppm (742-764 mg/kg bw/day) and the parental NOAEL is 1000 ppm (74-87 mg/kg bw/day) based on reduced body weight gain and reduced thymus weight in females at 10000 ppm (764-871 mg/kg bw/day).

The applicant was asked to submit histopathological data of the thymus from multigeneration study and the evaluation of these data was presented in the addendum. The NOAEL of the study was discussed at the experts' meeting. The NOAEL for developmental effects in the rat multigeneration study is 1000 ~~mg/kg bw/d~~ ppm (171 mg/kg bw/day) based on effects observed at 10000 ppm (1625 mg/kg bw/day) was agreed on at the experts' meeting.

In the rabbit developmental toxicity study, there was evidence for a slight delay in fetal development (slight dilation of lateral brain ventricles) at the top dose in the presence of severe maternal toxicity. There was also questionable evidence for a slight substance-related increase in the incidence of a common rib cartilage malformation and equivocal evidence for a slight increase in the incidence of one rib variation.
The NOAEL for maternal toxicity in the rabbit teratogenicity study is 25 mg/kg bw/day and the developmental is 100 mg/kg bw/day.

In the rat developmental toxicity study there was no substance-related adverse maternal or developmental effect. The reduction in ossification of one digit from both forelimbs of fetuses at 300 and 1000 mg/kg bw/day is not considered to be a substance-related adverse effect. The maternal and developmental NOAEL is 1000 mg/kg bw/day in rats.

Overall, it is concluded that fluoxastrobin is not teratogenic and that adverse developmental effects could be a consequence of substance-related parental toxicity. Classification of fluoxastrobin for reproductive toxicity is not justified.

CA 5.6.1 Generational studies

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.6.2 Developmental toxicity studies

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.7 Neurotoxicity studies

Summary neurotoxicity

Acute and subchronic neurotoxicity studies summarized in Table 5.7-1 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008).

Table 5.7-1: Summary of neurotoxicity studies

Study Doses tested	Sex	NO(A)EL ppm (mg/kg bw/day)	LO(A)EL ppm (mg/kg bw/day)	Main findings at LO(A)EL	Reference
Acute oral neurotoxicity, rat 0-200-500-2000 mg/kg bw) (99:1% *E:Z*)	M F	2000 2000	-- --	No neurotoxicity or general systemic toxicity observed up to the limit dose of 2000 mg/kg bw.	[redacted]; 2001; M-088080-01-1
Subchronic oral neurotoxicity, rat (diet) 0-200-1000-7500 ppm (M/F: 0-13/15-60/72-474/582 mg/kg bw/d) (99:1% *E:Z*)	M F	1000 (60) 1000 (72)	7500 (474) 7500 (582)	Reduced body weight Reduced body weight No evidence of substance-related neurotoxicity.	[redacted]; 2002; M-074246-01-1

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on neurotoxicity:

Fluoxastrobin gave a negative result with rats in an acute neurotoxicity assay which included neuropathology and a functional observation battery. There was also no evidence of substance-related neurotoxicity in a subsequent subchronic neurotoxicity assay in rats.

CA 5.7.1 Neurotoxicity studies in rodents

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR, addenda and the baseline dossier of fluoxastrobin.

CA 5.7.2 Delayed polyneuropathy studies

No data submitted. Since fluoxastrobin is not a member of a chemical class associated with delayed neurotoxicity and since there is no evidence of changes in nervous tissues, testing for delayed neurotoxicity is not required.

CA 5.8 Other toxicological studies

Summary toxicity studies of metabolites

Toxicity studies on metabolites summarized in the table below were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008), new studies are added.

Table 5.8-1: Summary of studies with metabolites

Test item,	Test System	Concentration/ Dose	Results	Reference
HEC 5725-des-chlorophenyl (M48)	Ames test	Up to 5000 µg/plate	Negative	[redacted]; 2003; M-105288-01-1
	HPRT (V79 cells)	Up to 3520 µg/plate	Negative	[redacted]; 2004; M-123365-01-1
	Chromosome aberration	Up to 3500 µg/mL Up to 450 µg/mL (pre-incubation)	Negative	[redacted]; 2004; M-123340-01-1
2-chlorophenol (M82)	*in vivo* rat absorption, metabolism & excretion study	5 mg/kg bw	rapid & complete absorption, conjugation & hydroxylation, very fast renal excretion	[redacted]; 2002; M-041282-01-1
	Ames test	Up to 3000 µg/plate	Negative	[redacted]カ; 2016; M-539465-01-1
	Micronucleus test *in vitro*	Up to 1000 µg/mL (4 h treatment) Up to 500 µg/mL (24 h treatment)	Positive	[redacted]カ; 2016; M-539476-01-1
	Micronucleus test *in vivo* (mouse)	2 x 0.06 mL/kg 2 x 0.30 mL/kg	Negative	[redacted]E; [redacted]; 1980; M-538343-01-2
	Micronucleus test *in vivo* (mouse)	2 x 0.06 mL/kg 2 x 0.30 mL/kg	Negative	[redacted],,[redacted]; 1980; M-538349-01-2
	Micronucleus test *in vivo* (rat)	Study ongoing		[redacted]カ; 2016; M-539480-01-1

HEC 5725-des-chlorophenyl (M48)

DAR section B.6.8.1, and Addendum 1 to the DAR:

M48 (E-isomer) is a prominent metabolite of fluoxastrobin in the rat, being found at up to 15% of the applied dose (being found mostly in faeces and bile, but also in urine at up to 4% of applied dose). It is also considered to be an initial metabolite and so the amount of M48 formed in the rat is likely to be greater than 15%. M48 is formed from fluoxastrobin by cleavage of the ether bridge between the chlorophenyl ring and the pyrimidine ring.

M48 has no structural alerts for DNA reactivity according to the model of Tennant and Ashby (1991) and was found to be negative when tested in an Ames assay conducted to modern standards. Additional reassurance that M48 is not genotoxic is provided by the genotoxicity assays with parent, all of which were negative. Since M48 is regarded as an initial metabolite of fluoxastrobin in rats, the negative result in an *in vivo* mouse bone marrow assay is particularly notable although it is

acknowledged that there might have been only transient, low-level exposure of the bone marrow to M48.

According to the "Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive" (SANCO/221/2000 –rev.10 –final, 25 February 2003) HEC5725-des-chlorophenyl (M48) was screened for genotoxicity in an Ames test, a gene mutation test with mammalian cells, and a chromosome aberration test. All tests led to negative results. Thus, HEC5725-des-chlorophenyl (M48) is considered to be non-genotoxic. The refined risk assessment for consumers has been performed, based on the ADI of fluoxastrobin ([redacted]; [redacted]; [redacted]; 2004; M-128831-01-1).

Further metabolites

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on wheat metabolites:

The following metabolites have been identified in wheat but not in rat metabolism

M34 = HEC 5725 – ketone
M39 = HEC 5725- CA –glycol ester
M40 = HEC 5725 - carboxylic acid
M41 = HEC 5725 - OH-CA + (M42 = glycosides of M41)
M57 = HEC 5725 - OH-phenoxy-amine PMD
M70 = HEC 5725-des-chlorophenyl-glycol-MA + (M71 = glycoside of M70)
M72 = HEC 5725-des-chlorophenyl-carboxylic acid
M82 = 2-chlorophenol + (M84 = glycoside of M82)

No genotoxicity tests, neither *in vivo* nor *in vitro* or acute toxicity test, were provided to define their toxicity. Therefore, they should be considered as toxicologically relevant and the ADI for fluoxastrobin used in the consumer risk assessment, unless new data are made available.

Confirmatory data related to residues were evaluated by the RMS UK and the assessment was made available with Addendum 8 to the DAR (initially January 2011 and a revised and updated version, April 2012). After assessment of the confirmatory data, the revised review report (SANCO/3921/07 dated 28 September 2012) was issued.

In order to address concerns raised during the EU review of fluoxastrobin regarding the toxicity of metabolites in cereal straws, the applicant provided a statement ([redacted]; [redacted]; [redacted]; [redacted]; 2008; M-257625-02-1). This statement addressed the potential toxicity of metabolites along with an estimate of the actual exposure of livestock to these metabolites, based on data from metabolism studies and residue field trials submitted in the DAR for Annex 1 inclusion.

….

With the exception of metabolites 2-chlorophenol (M82) and its glucoside (M84), the calculated residue levels for all other metabolites are below the toxicological trigger value of 0.05 mg/kg for raw animal fodder. Levels of these metabolites in straw can therefore be said not to be of concern based on the proposed uses.

2-Chlorophenol (M82)

The toxicokinetic behaviour of the metabolite 2-chlorophenol (M82) was investigated in a study on the absorption, metabolism and excretion in male rats after a single oral dose of [phenyl-UL-^{14}C]2-chlorophenol at 5 mg/kg bw ([redacted]; 2002; M-041282-01-1). 2-Chlorophenol was rapidly and completely absorbed from the gastrointestinal tract. Excretion was very fast and occurred almost exclusively with the urine. Already within 4 hours after dosage about 81% of the dose was excreted renally. Faecal excretion was minor (2.2% of the dose). The overall excretion was fast and nearly complete during the test period of 72 hours. Very low radiolabelled residues were found in the GIT,

carcass and skin at sacrifice (0.06% of the dose). 2-Chlorophenol was extensively metabolised in the rat, mainly by conjugation with glucuronic acid and sulfate. Hydroxylation was a minor pathway. Major metabolites were 2-chlorophenol-GA (M85, 63.6% of the dose) and 2-chlorophenol-SA (M83, 28.2% of the dose). 2-Chlorophenol-OH-GA (M86, 2.3% of the dose) and probably 2-chlorophenol-OH-SA (M87, 1.1% of the dose) were minor metabolites. The excreted portion of the test compound 2-chlorophenol was low (3.7% of the dose).

The genotoxic potential of 2-chlorophenol was investigated in *in vitro* and *in vivo* tests. 2-chlorophenol did not induce mutations in bacteria with and without metabolic activation but showed micronucleus formation in V79 cells. In two micronucleus tests in mice 2-chlorophenol revealed no genotoxic potential *in vivo*. In total 4 batches of 2-chlorophenol were tested, all resulted negative. However, the studies were conducted in 1980 not according to current guidelines and therefore, a new micronucleus test according to current testing guidelines was initiated in order to confirm these results.

E:Z isomerism

DAR section B.6.8.1:

Metabolites with the methoxyimino group have the potential to undergo E:Z isomerism. This process is aided by the presence of light. A 28-day toxicity study indicated that changing from a 98%E:2%Z to a 64%E:36%Z isomer ratio has no effect on the toxicity profile . . . It is therefore assumed that a similar change in the isomer ratio for metabolites of fluoxastrobin would have no effect on their toxicity.

No Z isomer metabolites have been detected in rat metabolism studies with HEC 5725 (E-isomer) but plant metabolites M03, M04, M07 and M48 have been identified as both E and Z isomers. However as the highest proportion of a Z isomer found to be present for a plant metabolite was not much higher than the 36%Z used in the 28-day toxicity study with HEC 5725, no significant effect on toxicity is expected.

CA 5.8.1 Toxicity studies of metabolites

2-chlorophenol (M 82)

In the "Reasoned opinion on the review of the existing maximum residue levels (MRLs) for fluoxastrobin …", EFSA Journal 2012;10(12):3012 further information about the toxicity of metabolites M82 and M84 found in straw is required. Genotoxicity studies according to modern standards are not available in the public literature. Therefore, the genotoxicity potential of 2-chlorophenol (M82) has been further investigated in *in vitro* and *in vivo* tests.

Report:	KCA 5.8.1/08[redacted]; 2016; M-539465-01-1
Title:	2-Chlorophenol - Salmonella typhimurium reverse mutation assay
Report No.:	AT06948
Document No.:	M-539465-01-1
Guideline(s):	Council Regulation No. 440/2008, B.13/14, OECD 471, US-EPA 712-C-98-247 OPPTS 870.5100
Guideline deviation(s):	none
GLP/GEP:	**yes**

I. Materials and methods

A. Materials

1. Test material:

Name:	2-Chlorophenol
Synonym:	AE C505780
CAS No:	95-57-8
Description:	Colourless liquid
Lot/Batch no:	SES 12956-2-1
Purity:	100.0% (w/w)
Stability of test compound:	guaranteed for study duration; expiry date: 2015-10-09

2. Vehicle and/or positive control: Vehicle: DMSO

Positive controls without metabolic activation:
TA1535, TA100: Sodium azide (NaN_3) in phosphate buffer
TA1537: 4-nitro-1,2-phenylene-diamine (4-NPDA) in DMSO
TA98: 2-nitrofluoren (4-NF) in DMSO
TA102 : mitomycin C (MMC) and cumene hydroperoxide (Cumene) in phosphate buffer
With metabolic activation :
TA1535, TA1537, TA98, TA100, TA102:
2-aminoanthracene (2-AA) in DMSO

3. Test system: *Salmonella typhimurium* TA98, TA100, TA102, TA1535, TA1537

Metabolic activation: S9 fraction prepared from livers of Aroclor 1254 induced male Sprague Dawley rats (protein content 22.3 mg per ml). Each batch checked for its metabolizing capacity and for possible contaminations.
Standard S9 mix: 8 mM/L MgCl2, 33 mM/L KCl, 5 mM/L glucose-6-phosphate, 4 mmol/L NADP, 100 mM/L sodium phosphate, pH 7.4, S9 at a concentration of 0.1 mL/mL of the mix.

Pre-cultures: Cultures of all organisms were prepared by overnight incubation (shaking at approximately 37 °C) of freshly inoculated nutrient broth. For inoculation, stock cultures which were stored at ca. –75 °C were used.

B. Study design and methods

1. Treatment

Dose:	Test item concentrations:	
	10-25-50-160-500-1600-5000 µg/plate (+/- S9-mix)	
	Positive controls:	
	NaN_3:	5 µg/plate (TA1535, TA100)
	4-NPDA :	10 µg/plate (TA1537)
	2-NF :	10 µg/plate (TA98)
	MMC :	0.2 µg/plate (TA102)
	Cumene :	50 µg/plate (TA102)[a]
	2-AA:	3 µg/plate + S9 mix (all strains)
		6 µg/plate + S9 (TA 102)
	For each test solution or control 3 plates were used.	
	[a]: only in preincubation trials	
Application volume:	0.05 mL (test solution)/plate	
Incubation time / temperature:	TA102: 37°C, 48h; TA1535, TA100, TA1537, TA98: 72h	

II. Results and discussion

A. Bacteriotoxicity, precipitation and controls

Titer determinations demonstrated sufficient bacterial density in the suspension, leading to spontaneous revertant frequencies that matched the ranges of the Historical Controls.

The Salmonella/microsome plate incorporation test, employing doses of up to 5000 µg per plate, showed the test item produced bacteriotoxic effects at the dose of 5000 µg per plate.

Substance precipitation was not observed. Evaluation of individual dose groups, with respect to relevant assessment parameters (dose effect, reproducibility) revealed no biologically relevant variations from the respective solvent controls.

The Salmonella/microsome test, using preincubation for 20 minutes at 37 °C and employing doses of up to 5000 µg per plate, showed the test item to produce strain-specific bacteriotoxic effects at the dose of 1600 µg per plate up to the highest dose. Substance precipitation was not observed. In agreement with the plate incorporation assay, evaluation of individual dose groups of the preincubation assay, with respect to relevant assessment parameters (dose effect, reproducibility), revealed no biologically relevant variations from the respective solvent controls.

B. Mutant counts

Table 5.8.1-1: Summary of mean values (mutant counts) without S9 Mix

Group	µg/plate	Strain TA1535	TA100	TA1537	TA98	TA102
Plate incorporation test						
2-Chloro-	0	8	108	7	27	238
phenol	10	11	109	7	30	235
	25	10	109	12	27	235
	50	9	123	10	29	214
	160	8	117	10	28	230
	500	10	116	9	34	212
	1600	7	80	7	19	198
	5000	0	22	0	0	160
Na-azide	5	336	394			
4-NPDA	10			166		
2-NF	10				593	
Cumene	50					426
MMC	0.2					347
Preincubation test						
2-Chloro-	0	13	113	11	30	215
phenol	10	11	111	11	31	215
	25	10	111	12	31	208
	50	7	126	12	31	204
	160	13	107	12	31	213
	500	11	107	9	35	223
	1600	2	1	3	13	112
	5000	0	1	0	1	0
Na-azide	5	171	284	-	-	-
4-NPDA	10	-	-	144	-	-
2-NF	10	-	-	-	1056	-
Cumene	50	-	-	-	-	354
MMC	0.2	-	-	-	-	349

- : not evaluated

Table 5.8.1-2: Summary of mean values (mutant counts) with S9 mix

Group	µg/plate	Strain TA1535	TA100	TA1537	TA98	TA102
Plate incorporation test						
2-Chloro-	0	12	127	12	24	242
phenol	10	11	129	11	21	248
	25	10	115	10	24	244
	50	10	123	7	20	243
	160	11	126	9	22	258
	500	14	127	7	26	276
	1600	11	102	6	24	252
	5000	7	84	0	2	36
2-AA	3	91	2060	260	1134	517
	6	-	-	-	-	1210
Preincubation test (µg/Plate)						
2-Chloro-	0	14	133	11	33	258
phenol	10	12	119	11	28	239
	25	13	134	15	29	245
	50	12	125	14	24	283
	160	14	126	12	33	293
	500	12	129	8	32	272
	1600	9	99	4	23	232
	5000	0	0	0	0	0
2-AA	3	99	1980	394	1511	534
	6	-	-	-	-	1096

- : not evaluated

The positive controls sodium azide, 4-nitro-1,2-phenylene-diamine, 2-nitrofluoren, mitomycin C, cumene hydroperoxide and 2-aminoanthracene increased mutant counts in the low doses used to well over those of the solvent controls, and thus demonstrated the system's sensitivity and the activity of the S9 mix.

None of the five strains used showed a dose-related and biologically relevant increase in mutant counts caused by 2-chlorophenol over those of the solvent controls in the plate incorporation test. This applied both to the tests with and without S9 mix and was confirmed by the results of the preincubation trials.

Despite this sensitivity no indications of mutagenic effects of the test item could be found at doses of up to 5000 µg per plate in any of the Salmonella typhimurium strains used in the plate incorporation assay as well as in the preincubation modification.

III. Conclusions

Due to these results 2-chlorophenol has to be regarded as non-mutagenic.

Report: KCA 5.8.1/09 [redacted] ; 2016; M-539476-01-1
Title: 2-Chlorophenol - In vitro micronucleus test with Chinese hamster V79 cells
Report No.: AT06947
Document No.: M-539476-01-1
Guideline(s): OECD 487 (2010)
Guideline deviation(s): none
GLP/GEP: **yes**

I. Materials and methods

A. Materials

1. Test material:

Name:	2-Chlorophenol
Synonym:	AE C505780
CAS No:	95-57-8
Description:	Colourless liquid
Lot/Batch no:	SES 12956-2-1
Purity:	100.0% (w/w)
Stability of test compound:	guaranteed for study duration; expiry date: 2015-10-09

2. Vehicle and positive control: Vehicle: DMSO
Mitomycin C in culture medium
Cyclophosphamide in culture medium
Vinblastine sulfate salt in DMSO

3. Test system: Chinese hamster V79 cells

Metabolic activation: S9 fraction prepared from livers of Aroclor 1254 induced male Sprague Dawley rats (protein content 22.9 mg per ml). Cofactor solution per 25 mL S9 mix: 40.7 mg $MgCl_2x6H_20$, 61.5 mg KCl, 38 mg glucose-6-phosphate, 4 78.8 mg NADP (disodium salt), 15 mL sodium phosphate buffer (100 mM; pH 7.4), final concentration of 2% S9 in cultures.

Culturing of V79 cells: Thawed stock cultures were propagated at 37 °C and 5 % CO_2 in plastic flasks:
Culture medium: MEM (Earle's with GlutaMAX and 25 mM HEPES), 1% Pen/Strep, 10% FBS
Cells were sub-cultured twice weekly after trypsination of adherently growing cells, ensuring an ample number of viable cells for the experiments performed in this study.

B. Study design and methods

1. Treatment

Dose: Test item concentrations:
Pre-test: 0.1-0.5-1-5-10-50-100-162.5-325-650-1300 µg/mL (4 h with and without metabolic activation, 24 h without metabolic activation)
Main study:
4 h treatment: 15-50-150-300-450-600-750-1000 µg/mL (+/- S9-mix)
24 h treatment: 0.5-1-2.5-5-10-25-50-100-150-200-250-500 µg/mL (- S9-mix)
Positive controls:
Mitomycin C: 0.1 µg/mL (4 h treatment)
Cyclophosphamide: 2 µg/mL (4 h treatment)
Vinblastine sulfate salt: 0.002 µg/mL(24 h treatment)

Application volume: 0.05 mL(test solution)/5 mL culture

Evaluation on micronucleated cells: Adherently growing cells exposed in situ to 0.4% KCl hypotonic solution and fixed in glacial acetic acid/ethanol (1+3), staining with May-Grünwald and Giemsa solutions.
2000 cells (1000 cells per slide) per concentration were scored. Only cells which divided at least once and, therefore, formed colonies of ≥ 2 cells were evaluated.

Assessment criteria:

The test item is classified as mutagenic if:

- one of the test substance concentrations induces a micronucleus frequency that is three times higher than the micronucleus frequency of the concurrent solvent control.
- there is a reproducible concentration-related increase in the micronucleus frequency. Such an evaluation may be considered independently of the enhancement factor for induced micronucleus frequencies.

In the evaluation of the test results historical control data, obtained in the laboratory and scientific plausibility is taken into consideration.
Any positive test result should be evaluated for its biological relevance.

II. Results and discussion

A. General Remarks

The test item, dissolved in DMSO, was examined for mutagenic activity in the micronucleus test in vitro. The 4 hours treatment was conducted with concentrations of 15 - 1000 µg/mL without S9 mix and of 15 - 1000 µg/mL with S9 mix. In the independent repeat test, the treatment time in the experiment without S9 mix was extended to 24 hours with concentrations of 0.5 - 500 µg/mL.

B. Cytotoxicity

Without S9 mix cytotoxic effects occurred at 600 µg/mL and above after 4 hours treatment and at 50 µg/mL and above after 24 hours treatment. With S9 mix cytotoxic effects were observed at 15 µg/mL and above. Precipitation in the medium did not occur.

C. Main study

Concentrations of 150 - 600 µg/mL (without S9 mix, 4 hours treatment) and 150 - 450 µg/mL (with S9 mix, 4 hours treatment) were chosen for reading. Higher concentrations were excluded from evaluation for micronuclei due to excessive cytotoxicity.

The repeat experiment (24 hours treatment without S9 mix) was not evaluated for proliferation index and micronucleus frequency based on the results of the experiments with 4 hours treatment.

Solvent control (dimethyl sulfoxide) and appropriate positive controls with known mutagens (mitomycin C, cyclophosphamide) demonstrated the suitability and sensitivity of the test system.

Table 5.8.1-3: Summary of results of 4 hours treatment (24 h harvest)

Experimental Group	Concentration	S9 Mix	Cytotoxicity		1-5 MN [1]	≥ 6 MN
			RICC [2]	PI [3]		
	µg/mL	-/+	Mean %	Mean %	Mean %	Mean %
Solvent control	1 % (v/v)	-	0	0	0.6	0.0
2-Chlorophenol	150	-	16	6	0.4	0.0
	300	-	34	12	1.1	0.0
	600	-	57	36	3.3	0.0
Mitomycin C	0.1	-	not tested	30	13.2	0.0
Solvent control	1 % (v/v)	+	0	0	1.0	0.0
2-Chlorophenol	150	+	38	20	6.0	0.0
	300	+	39	33	5.9	0.0
	450	+	58	41	6.7	0.0
Cyclophosphamide	2.0	+	not tested	57	13.7	0.0

[1]: MN = Micronuclei; [2]: RICC = Relative increase in cell count; [3]: PI = Proliferation index

III. Conclusions

In conclusion, it can be stated that under the reported experimental conditions the test item did induce chromosome breakage (structural chromosomal aberrations) or misdistribution of chromosomes leading to micronucleus formation in V79 cells in vitro either in the absence or presence of metabolic activation.

Report:	KCA 5.8.1/10[redacted],;[redacted], 1980; M-538343-01-2
Title:	Study of the possible mutagenic potential of ortho-monochlorophenol (containers 5244 to 5357 of February 1980) in the mouse by the micronucleus technique
Report No.:	445
Document No.:	M-538343-01-2
Guideline(s):	not specified
Guideline deviation(s):	none
GLP/GEP:	**no**

I. Materials and methods

A. Materials

1. Test material:

Name:	Ortho-monochlorophenol
Synonym:	2-chlorophenol
Description:	Colourless liquid
Lot/Batch no:	Containers 5244 to 5357 of 12 February 1980
Purity:	Not stated
Stability of test compound:	Not stated

2. Vehicle / positive control: Vehicle: peanut oil

Ortho-monochlorophenol dissolved in peanut oil dissolved in a concentration of 2.4 and 12.0 µL/mL

Positive control: Methanesulfonate (MMS) soluble in water dissolved in a concentration of 2 µL/mL

Cisplatin suspended in peanut oil at a concentration of 0.4 mg/mL

3. Test animals

Species:	Mice
Strain:	Swiss C.I.L.P.([redacted]) strain
Age:	8 - 11 weeks
Weight at dosing:	Males: 25-30 g
Source:	Not stated
Acclimatisation period:	Not stated
Diet:	Not stated
Water:	Not stated
Housing:	Not stated

B. Study design and methods

1. Animal assignment and treatment

Dose:	Negative control:	peanut oil	2 x 25 mL/kg
	Ortho-monochlorophenol	peanut oil	2 x 0.06 mL/kg
	Ortho-monochlorophenol	peanut oil	2 x 0.30 mL/kg
	Positive control:		
	MMS	water	2 x 65 mg/kg
	Cisplatin	peanut oil	2 x 10 mg/kg
Application route:	Oral, gavage		
Application volume:	25 mL/kg bw 2x 24 hours apart		
Group size:	10 per dose group		
Observations:	mortality, clinical signs		
No. of cells scored:	Not stated		

2. Evaluation

Method:	According to SCHMID published by BOLLER and SCHMID (1970) and SCHMID (1975)
Statistics:	Comparison of two means carried out using the Student's t-test which is valid for small samples.

II. Results and discussion

A. Clinical observations

The dose of 2 x 0.30 ml ortho-monochlorophenol per kg body weight caused signs of severe prostration in the animals. The dose was considered to be the maximum dose in this experiment.

B. Microscopic Evaluation

The mean percentages of micronucleated polychromatophilic erythrocytes are not statistically significantly increased in the groups of animals treated with ortho-monochlorophenol compared to the mean percentages in the control group.

In contrast, the mean percentages of micronucleated polychromatophilic erythrocytes obtained in the groups treated with MMS or cisplatin, used as positive controls, increased statistically significantly ($p < 0.05$) compared to the mean percentages in the control group.

Table 5.8.1-4: Summary of results

Experimental groups	Percentage of micronucleated P.E. Mean + 2SD
Negative control peanut oil	0.25 ± 0.08
Ortho-monochlorophenol 2x 0.06 mL/kg	0.28 ± 0.06
Ortho-monochlorophenol 2x 0.30 mL/kg	0.31 ± 0.07
MMS 2x 65 mg/kg	2.60 ± 0.60 *
Cisplatin 2x 10 mg/kg	2.56 ± 0.85 *

* Statistically significant different from control p < = 0.05

III. Conclusions

Exposure of ortho-monochlorophenol to mice via the oral route at doses of 2x 0.06 and 2x 0.30 ml/kg did not cause an increase in the percentage of micronucleated polychromatophilic erythrocytes.

Report:	KCA 5.8.1/11 [redacted],; [redacted]; 1980; M-538349-01-2
Title:	Study of the possible mutagenic potential of ortho-monochlorophenol (containers 5759 to 5788 of 17 March 1980) in the mouse by the micronucleus technique
Report No.:	446
Document No.:	M-538349-01-2
Guideline(s):	not specified
Guideline deviation(s):	none
GLP/GEP:	**no**

I. Materials and methods (see M-538343-01-2 for all study details)

A. Materials

1. Test material:

Name:	Ortho-monochlorophenol
Synonym:	2-chlorophenol
Description:	Colourless liquid
Lot/Batch no:	Containers 5759 to 5788 of 17 March 1980
Purity:	Not stated
Stability of test compound:	Not stated

II. Results and discussion

A. Clinical observations

The dose of 2 x 0.30 ml ortho-monochlorophenol per kg body weight caused signs of severe prostration in the animals. The dose was considered to be the maximum dose in this experiment.

B. Microscopic Evaluation

The mean percentages of micronucleated polychromatophilic erythrocytes are not statistically significantly increased in the groups of animals treated with ortho-monochlorophenol compared to the mean percentages in the control group.

In contrast, the mean percentages of micronucleated polychromatophilic erythrocytes obtained in the groups treated with MMS or cisplatin, used as positive controls, increased statistically significantly ($p < 0.05$) compared to the mean percentages in the control group.

Table 5.8.1-5: Summary of results

Experimental groups	Percentage of micronucleated P.E. Mean + 2SD
Negative control peanut oil	0.25 ± 0.08
Ortho-monochlorophenol 2x 0.06 mL/kg	0.19 ± 0.05
Ortho-monochlorophenol 2x 0.30 mL/kg	0.18 ± 0.05
MMS 2x 65 mg/kg	2.60 ± 0.60 *
Cisplatin 2x 10 mg/kg	2.56 ± 0.85 *

* Statistically significant different from control p < = 0.05

III. Conclusions

Exposure of ortho-monochlorophenol to mice via the oral route at doses of 2x 0.06 and 2x 0.30 ml/kg did not cause an increase in the percentage of micronucleated polychromatophilic erythrocytes.

Publications

Report:	KCA 5.8.1/13 [redacted]; 2013; M-486887-01-1
Title:	Comparative susceptibility of newborn and young rats to six industrial chemicals
Report No.:	M-486887-01-1
Document No.:	M-486887-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KCA 5.8.1/14 [redacted]; 2013; M-486888-01-1
Title:	Pediatric susceptibility to 18 industrial chemicals: a comparative analysis of newborn with young animals
Report No.:	M-486888-01-1
Document No.:	M-486888-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Abstract (*verbatim* from the publication):
"*To elucidate the comparative susceptibility of newborn rats to chemicals, newborn and young animals were administered six industrial chemicals by gavage from postnatal days (PND) 4 to 21, and for 28 days starting at 5–6 weeks of age respectively, under the same experimental conditions as far as possible. As two new toxicity endpoints specific to this comparative analysis, presumed no-observed-adverse-effect-levels (pNOAELs) were estimated based on results of both main and dose-finding studies, and presumed unequivocally toxic levels (pUETLs) were also decided. pNOAELs for newborn and young rats were 40 and 200 for 2-chlorophenol, 100 and 100 for 4-chlorophenol, 30 and 100 for p-(α,α-dimethylbenzyl) phenol, 100 and 40 for (hydroxyphenyl)methyl phenol, 60 and 12 for trityl chloride, and 100 and 300 mg/kg/day for 1,3,5-trihydroxybenezene, respectively. To determine pUETLs, dose ranges were adopted in several cases because of the limited results of experimental doses. Values for newborn and young rats were thus estimated as 200–250 and 1000 for 2-chlorophenol, 300 and 500 for 4-chlorophenol, 300 and 700–800 for p-(α,α-dimethylbenzyl) phenol, 140–160 and 1000 for (hydroxyphenyl)methyl phenol, 400–500 and 300 for trityl chloride, and 500 and 1000 mg/kg/day for 1,3,5-trihydroxybenzene, respectively. In most cases, newborn rats were 2–5 times more susceptible than young rats in terms of both the pNOAEL and the pUETL. An exception was that young rats were clearly more susceptible than their newborn counterparts for trityl chloride.*"

The results from this study have been re-evaluated in M-486888-01-1. Here, the susceptibility of young rats compared to newborn rats was assessed by the BMDL ratio. However, the susceptibility based on the BMDL ratio of young and newborn rats is in the same range as the pNOAEL ratio presented in the current publication, i.e. 4.1 and 4.0-5.0 (BMDLs/pNOAELs are 4.1/4-5 times higher in young rats than in newborn rats), respectively. Hence, the re-evaluation did not identify new relevant information.

I. Materials and methods

A. Materials

1. Test material: 2-Chlorophenol

Among 5 other chemicals (4-chlorophenol, p-(α,α-Dimethylbenzyl) phenol, (Hydroxyphenyl) methyl phenol, Trityl chloride, 1,3,5-Trihydroxybenzene), only the results for 2-chlorophenol are presented here.

CAS no.:	95-57-8
Description:	Not available
Lot/Batch no:	OJL-15
Purity:	99.49%
Stability of test compound:	No expiry data available. Test solutions were prepared at least once a week and were kept cool and in the dark until dosing. The stability was confirmed to be at least seven days under these conditions.
Source:	[redacted] Corporation

2. Vehicle: Olive oil

3. Test animals

Species:	Sprague-Dawley SPF rat
Strain:	Crj:CD(SD)IGS
Source:	[redacted], Japan)
Diet:	Not specified, *ad libitum*
Water:	Not specified, *ad libitum*
Housing:	Not specified
Test facility:	Environmentally controlled room at 24 ± 2°C with a relative humidity of 55% ± 15, a ventilation rate of more than 10 times per hour, and a 12-12 h light/dark cycle.

18-day repeated dose study in newborn rats ("newborn study")

Sex:	Female, pregnant - gestation day 14
Age:	Not specified
Details:	20 pregnant rats (shipped in at gestation day 14) were allowed to deliver spontaneously. All newborns were separated from dams on postnatal day (PND) 3 and groups of 12 males and 12 females were selected and assigned to each of the four dose groups, including the controls. Twelve foster mothers were selected based on health and nursing conditions, and suckled the four males and four females assigned to each group up to weaning on PND 21 (termination of dosing and autopsy for half of the animals). After weaning, the rest of the animals for the recovery-maintenance group (see Study Design) were individually maintained for nine weeks.

28-day repeated dose study in newborn rats ("newborn study")

Sex:	Male and female
Weight:	Not specified
Age:	4-weeks old
Acclimatization:	Used at ages of 5–6 weeks after acclimation

B. Study design and methods

1. Animal assignment and treatment

18-day repeated dose study in newborn rats ("newborn study")

Dose*:	Dose-finding study:	0, 20, 100, 500 mg/kg bw
	Main study:	0, 8, 50, 300 mg/kg bw
Treatment period:	Dose-finding study:	PND 4-21
	Main study:	PND 4-21
Application route:	Gastric intubation	
Application volume:	Not specified	
Fasting time:	See observations	
Group size:	Dose-finding study:	4/group
	Main study:	12/group
Post-treatment observation period:	Main study: 9 weeks for the recovery groups (half of the animals of the main study)	
Observations:	Dose-finding study: general behavior, body weight, hematology, blood biochemistry, macroscopic findings, organ weights.	

Main study: general behavior and body weight at least once a day and each week, respectively. Surface righting and visual placing reflex for reflex ontogeny, fur appearance, incisor eruption, eye opening, preputial separation, vaginal opening and estrous cycle. Urinalysis (color, pH, occult blood, protein, glucose, ketone bodies, bilirubin, urobilinogen, sediment, volume of the urine and osmotic pressure) was conducted in the late recovery-maintenance period.

At weaning age, PND 22 after the last treatment, blood was collected under anesthesia from the abdomen of all animals in the scheduled-sacrifice group. In the recovery-maintenance group, this was conducted at 85 days of age after overnight starvation. Blood was examined for hematological parameters such as the red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, reticulocyte count and differential leukocyte count, and for biochemistry (total protein, albumin, albumin/globulin ratio, glucose, total cholesterol, triglycerides, phospholipid, total bilirubin, urea nitrogen (BUN), creatinine, aspartate aminotransferase, alanine animotransferase (ALT), alkaline phosphatase, γ-glutamyl transpeptidase (γ-GTP), calcium, inorganic phosphorus, sodium, potassium and chlorine).

Prothrombin time and activated thromboplastin time were examined only in the recovery-maintenance group. The brain, pituitary gland, thymus, thyroids, heart, lungs, liver, spleen, kidneys, adrenals, testes, epididymides, ovaries and uterus were weighed, and these, with other macroscopically abnormal organs, were fixed in 10% buffered formalin-phosphate (following Bouin's fixation for testes and epididymides). Paraffin sections were routinely prepared and stained with hematoxylin-eosin for microscopic examination.

All studies were conducted in compliance with the Good Laboratory Practice Act of the Japanese Government.

28-day repeated dose study in newborn rats ("young study")

Dose*:	Dose-finding study:	0, 100, 200, 500 mg/kg bw
	Main study:	0, 100, 200, 1000 mg/kg bw
Treatment period:	Dose-finding study:	14 days
	Main study:	28 days
Application route:	Gastric intubation	
Application volume:	Not specified	
Group size:	Dose-finding study:	3/group
	Main study:	12/group
Post-treatment observation period:	Main study: 14 days for the recovery groups (half of the animals of the main study)	
Observations:	Dose-finding study: general behavior, body weight, food consumption hematology, blood biochemistry, macroscopic findings and organ weights. Main study: general behavior, body weight, food consumption, urinalysis, hematology and blood biochemistry, necropsy findings, organ weights and histopathological findings	

BCS Comment: ***Used doses were in the toxic range. Harmonized classification: Acute Tox 4, H302, H312, H332; $LD_{50\ [rat]}$ ~ 600-700 mg/kg bw***
Used doses are ambiguously stated. The investigated doses are not given in the materials and methods section but have to be derived from the results section.
** effects of underlined doses are given in Table 1.*

2. Statistics

Quantitative data were analyzed by [redacted] test ([redacted] 1937) for homogeneity of distribution. When homogeneity was recognized, [redacted] test ([redacted] 1964) was conducted for comparison between control and individual treatment groups. If not homogenous, the data were analyzed using Steel's multiple comparison test ([redacted] 1959) or the mean rank test of the [redacted] type ([redacted] 1973).

For qualitative data such as histopathological findings, the [redacted] *U*-test ([redacted] 1947) or the [redacted] exact test ([redacted] 1973) were performed.

BCS Comment: ***Based on the result of [redacted] Test, data were either submitted to [redacted] test that assumes normal data or [redacted] multiple comparison test that does not assume normal data. However, both tests assume similar variances between groups, which have not been assessed in the performed statistical analyses. It is unclear whether groupwise comparisons were performed with the U-test and if alpha was sufficiently corrected. Hence the Type I error rate might not be sufficiently controlled (resulting in an increased rate of "false positive" statistical findings).***

II. Results and discussion

A. Mortality

All newborn animals treated with 500 mg/kg in the dose-finding study died by the 9th dosing day.

The newborn investigation was conducted at doses of 0, 20, 100, and 500 mg/kg for the dose-finding and 0, 8, 50, and 300 mg/kg for the main study.

The young investigation was conducted at doses of 0, 100, 200, and 500 mg/kg for the dose-finding and 0, 8, 40, 200, and 1000 mg/kg for the main study.

Body weights of both sexes were only transiently, but not finally reduced, at 300 mg/kg in the newborn main study. Clinical signs in newborn rats were not observed at doses of 20 and 100 mg/kg in the dose-finding study.

Major toxic effects on the central nervous system (CNS) were found in both sexes of newborn and young rats. In the newborn study, tremors appeared within five minutes and disappeared within four hours in most animals at 300 mg/kg. Hypoactivity and an abnormal gait were also observed in a few cases. The histopathological examination showed slight to moderate basophilic renal tubules in more than half the animals of both sexes, without relative kidney weight changes (increase by 8% for males, 4% for females). In addition to these effects, the body weights of both sexes at this dose were transiently decreased. At 50 mg/kg, only one female showed tremors once from 15 to 30 minutes on day nine after the dosing start. There were no chemical-related changes in developmental parameters. In the young study, most animals of both sexes sporadically showed various effects on the CNS such as tremors, hypoactivity, and an abnormal gait within three hours after dosing at 1000 mg/kg. Most animals also exhibited slight centrilobular hypertrophy of hepatocytes, suggesting a compensatory response to a requirement for hepatic metabolism. In the dose-finding study, no toxic signs were observed, but the information was limited because of the small number of animals, the short administration period, and the lack of histopathological examination. There were no chemical-related abnormalities at 200 mg/kg in the main study.

Although the NOAEL was 8 mg/kg/day for newborn rats based on the main study results, this value was concluded to be too low because of the absence of clinical signs at 20 and 100 mg/kg in the dose-finding study, and only one female showed tremors once at 50 mg/kg in the main study. The pNOAEL for newborn rats was therefore estimated to be 40 mg/kg/day, a little below the 50 mg/kg.

BCS Comment: The lack of a clear dose-response relationship, even with small animal numbers in the dose-finding study, apparently casts doubt on the reliability of the study. The pNOAEL is based on an isolated observation of 15 min tremor of a single female on treatment day 9.

For young rats, the pNOAEL can be considered to be 200 mg/kg/day because of the limited information at 500 mg/kg in the dosefinding study. The toxicity at 300 mg/kg for newborn rats seemed to be slightly higher than that at 1000 mg/kg for young rats, because of the transient depression of body weight found limited to the former cases, although the toxicity profile regarding the CNS was very similar in newborn and young rats. The dose for newborn rats showing the same toxic intensity, as that for young rats at 1000 mg/kg, is considered to be slightly lower than 300 mg/kg, at 200–250 mg/kg/day. Therefore, pUETLs of 200–250 and 1000 mg/kg/day may be considered equivalent doses for newborn and young rats, respectively.

Toxicity findings for 2-chlorophenol in the newborn and young rat main studies

	Newborn study (mg/kg)					Young study (mg/kg)			
	0	20†	50	100†	300	0	200	500†	1000
Male									
General behavior									
Tremors	0/12	0/4	0/12	0/4	11/12	0/12	0/12	0/3	4/12
Hypoactivity	0/12	0/4	0/12	0/4	2/12	0/12	0/12	0/3	3/12
Abnormal gait	0/12	0/4	0/12	0/4	1/12	0/12	0/12	0/3	4/12
Histopathology									
Renal tubules, basophilic	0/6	no data	0/6	no data	4/6	0/6	0/6	no data	0/6
Centrilobular hypertrophy	0/6	no data	0/6	no data	0/6	0/6	0/6	no data	0/6
Female									
General behavior									
Tremors	0/12	0/4	1/12	0/4	9/12	1/12	0/12	0/3	5/12
Hypoactivity	0/12	0/4	0/12	0/4	3/12	0/12	0/12	0/3	5/12
Abnormal gait	0/12	0/4	0/12	0/4	1/12	0/12	0/12	0/3	7/12
Histopathology									
Renal tubules, basophilic	0/6	no data	0/6	no data	5/6	0/6	0/6	no data	0/6
Centrilobular hypertrophy	0/6	no data	0/6	no data	0/6	0/6	0/6	no data	0/6

Only data for items showing change are included in this table. Data are numbers of animals with the change of the total examined. †indicates dose and data from the dose-finding study. All newborn animals died by the 5th dosing day at 300 mg/kg in the dose-finding study. Body weights of both sexes were only transiently, but not finally reduced, at 300 mg/kg in the newborn main study. Clinical signs in newborn rats were not observed at doses of 20 and 100 mg/kg in the dose-finding study.

III. Conclusion

According to the authors newborn rats seem to be 5-times more susceptible than young rats to the effects of 2-chlorophenol.

Reliability (Klimisch Score):	**Not reliable (Klimisch code 3)**
Details:	- no GLP, no guideline - body weight and age (maternal animals) not specified - diet and cage type not specified - application volume not stated - statistical analysis for qualitative data not well documented - study results mainly not reported (e.g. hematology, blood biochemistry, organ weights etc.)
Relevance:	**Not relevant**
Details:	- toxic dose was tested - results from pre-test were used to derive endpoints (small group size)
BCS Conclusion	This study does not satisfy important criteria used to judge data reliability and human relevance. The study results have no impact on the overall conclusion for the active substance fluoxastrobin.

CA 5.8.2 Supplementary studies on the active substance

Summary of supplementary studies on fluoxastrobin

Supplementary studies on the active substance summarized in Table 5.8.2-1 and Table 5.8.2-2 were evaluated in the EU peer review for inclusion of fluoxastrobin into Annex I of Directive 91/414/EEC (2008), new studies are added.

Table 5.8.2-1: Summary of supplementary studies on fluoxastrobin

Study Doses tested	Sex	NO(A)EL ppm (mg/kg bw/day)	LO(A)EL ppm (mg/kg bw/day)	Main findings at LO(A)EL	Reference
Mouse					
5-week immuno-toxicity mouse (diet) 0-450-1800-7000 ppm (M/F: 0-107/157-367/660-1543/2383 mg/kg bw/d) (99:1 *E:Z*)	M	7000 (1543)	--	No adverse effects in a plaque-forming cells assay	[redacted]; 2001; M-076778-01-1
	F	7000 (2383)	--		
Rat					
28-day oral (diet) male rat 0-125-1000-8000 ppm (0-10-81-637 mg/kg bw/d)	M	8000 (637)	--	Immunotoxicity no effect	[redacted]; 2011; M-441880-01-1
9-week mechanistic study in rats particularly of effects on urinary system (diet) 0-62.5/125-125/250-1000/2000-8000/16000 ppm (M/F: 0-4/9-7/18-60/146-520/1544 mg/kg bw/d) (99:1 *E:Z*)	M	125 (7)	1000 (60)	Effects on urine and phosphate and calcium homeostasis	[redacted]; 2001; M-136709-01-1 supplementary information/data: [redacted]; 2001; M-072428-01-1 [redacted]; 2001; M-081668-01-1
	F	2000 (146)	16000 (1544)	Effects on urine and phosphate and calcium homeostasis	

In the short-term toxicity studies summarised in document MCA 5.3 (M-012683-01-1, M-017457-01-1, M-012710-01-1) immunotoxicity investigations are included which showed no effect on immunotoxicity.

EFSA Scientific Report (2007) 102, 1-84, "Conclusion regarding the peer review of the pesticide risk assessment of the active substance fluoxastrobin finalised: 13 June 2007" on supplementary studies:

Immunotoxicity

Studies in rats and mice did not reveal any adverse immunotoxic effects following dietary exposure for 4-13 weeks to high doses. Two studies included the plaque forming cell assay. There was however no specific investigation of the thymus, an organ for which reduced weight was observed in adults and pups in the multigeneration study. The new immunotoxicity study in male rats (M-441880-01-1) confirmed that fluoxastrobin shows no immunotoxicity potential. Furthermore, there were no substance-related effects on spleen and thymus weights.

Calcium-phosphorous homeostasis

Following further mechanistic investigations, it was concluded that exposure to fluoxastrobin resulted in reduced phosphate absorption in the intestine. A potential phosphate deficiency was counter-regulated by reduced renal excretion of phosphate and renal hyper-excretion of calcium. It is proposed that increased calcium excretion in urine, together with an increase in urinary pH, led to calculi formation.

Summary of supplementary studies on impurities of fluoxastrobin

Table 5.8.2-2: Summary of supplementary studies with impurities of fluoxastrobin

Test item (purity)[#]	Type of study	Dose range tested	Results	Reference
Impurity 7 99.2%	Acute oral, rat	2000 mg/kg bw	LD_{50} >2500 mg/kg	[redacted]; 2002; M-066922-01-1
	Reverse mutation*	1st: 16-5000 µg/plate 2nd: 100-2500 µg/plate	Negative	[redacted]; 2002; M-073511-01-1
	In vitro HPRT	5-80 µg/mL (+/- S9-mix)	Negative	[redacted]; 2004; M-107900-01-1
	In vitro chromosome aberration	4h treatment: 20-80 µg/mL 18h treatment: 3-15 µg/mL	Negative	[redacted]; 2004; M-002507-01-1
Impurity 15 98.9%	Acute oral, rat	2000 mg/kg bw	LD_{50} >2500 mg/kg	[redacted]; 2003; M-075146-01-1
	Reverse mutation*	1st and 2nd:16-5000 µg/plate	Negative	[redacted]; 2002; M-073977-01-1
Impurity 20 99.1%	Acute oral, rat	2000 mg/kg bw	LD_{50} >2500 mg/kg	[redacted]; 2002; M-063677-01-1
	Reverse mutation*	1st and 2nd:16-5000 µg/plate	Negative	[redacted]; 2002; M-073957-01-1
Impurity 21 99%	Acute oral, rat	2000 mg/kg bw	LD_{50} >2500 mg/kg	[redacted]; 2003; M-075063-01-1
	Reverse mutation*	1st and 2nd:16-5000 µg/plate	Negative	[redacted]; 2002; M-073941-01-1
Impurity 22 98.6%	Acute oral, rat	2000 mg/kg bw	LD_{50} >2500 mg/kg	[redacted]; 2003; M-075100-01-1
	Reverse mutation*	1st: 16-5000 µg/plate 2nd: 51-1581 µg/plate	Negative	[redacted]; 2003; M-082964-01-1
Impurity 23 94.5%	Acute oral, rat	200 and 2000 mg/kg bw	LD_{50} >300 < 500 mg/kg	[redacted]; 2002; M-090369-01-1
Impurity 23 99.1%	Reverse mutation*	1st: 16-5000 µg/plate 2nd: 2-480 µg/plate	Negative	[redacted]か; 2002; M-036525-01-1

* Plate incorporation in first experiment, pre-incubation in second experiment

[#] Impurity denominations are deciphered in Document JCA.

Immunotoxicological studies

In order to fulfil US EPA requirements an additional immunotoxicity study in rats was conducted in 2011. The respective study M-441880-01-1 is owned by Arysta LifeScience (ALS). ALS provides a Letter of Access for the benefit of Bayer CropScience (see document M-532402-01-1).

Report:	KCA 5.8.2/22[redacted]; 2011; M-441880-01-1
Title:	Immunotoxicity evaluation of fluoxastrobin technical in a 28 day dietary study in Sprague Dawley CD male rats: Evaluation of anti-sheep red blood cell (SRBC)
Report No.:	BRT 20110205
Document No.:	M-441880-01-1
Guideline(s):	Health Effects Test Guidelines OPPTS 870.7800. Immunotoxicity
Guideline deviation(s):	not applicable
GLP/GEP:	**yes**

Report:	KCA 1.11/08[redacted]Z; 2005; M-532402-01-1
Title:	Fluoxastrobin - Letter of access
Report No.:	M-532402-01-1
Document No.:	M-532402-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

I. Materials and methods

A. Materials

1. Test material: Fluoxastrobin technical

Synonym(s):	HEC 5725
CAS No.:	361377-29-9
Lot/Batch no:	EDC 1003797
Purity:	99.2% w/w (Test substance in diet was adjusted for purity)
Stability of test compound:	Guaranteed for study duration; (stable for 33 days at room temperature in the diet)

2. Vehicle and positive control:

Vehicle:	Diet
Positive control:	15 mg/kg cyclophosphamide at 5 mL/kg, *intraperitoneal* (once daily on days 23-28)

3. Test animals

Species:	Rat
Strain:	Sprague Dawley CD
Sex:	Male
Age:	7 weeks
Weight at dosing:	188 - 228 g
Source:	[redacted], USA
Acclimatisation period:	7 days
Diet:	PMI® LabDiet (5002 Certified Rodent Diet) (pelleted), *ad libitum*

Water:	Tap water, *ad libitum*
Housing:	Group-housed (maximum of 3 rats per cage) upon receipt and individually housed during the dosing phase of the study. Animals were housed in individually ventilated plastic cages containing bedding material. The bedding (bed-o'cobs®) was changed at least once weekly. Each cage was labeled with the study number, animal number, and treatment regimen. Individual animals were identified by tail marking

B. Study design and methods

1. Animal assignment and treatment

Treatment:	0, 125, 1000, 8000 ppm (equivalent to approx 0, 10, 81, 637 mg/kg bw/day)
Application route:	Diet
Exposure:	28 days
Group size:	10 animals/group (50 rats in total)
Antigen stimulation:	Sheep red blood cell (SRBC) sensitization
Identification:	Sheep red blood cell (SRBC)
Source of SRBC:	[redacted], USA
Preparation of SRBC:	Pooled SRBC in Alsever's solution were washed 3 times in PBS and resuspended to a final concentration of 5×10^7 SRBC/mL.
Administration of SRBC:	Each rat was immunized intravenously into a tail vein with 0.2 mL of this preparation on Day 23.
Observations:	Body weight, food and water consumption, clinical observations, organ weights (spleen, thymus), IgM with an ELISA

2.Assays

Anti-SRBC ELISA:	Serum samples were evaluated with ELISA kit. Diluted test samples and standards were added to microwells and incubated for 45 minutes. The wells were washed and horseradish peroxidase-conjugated anti-rat IgM added to the wells. The microplate was incubated at room temperature for 45 minutes, the wells washed, and the substrate solution added. Color development was stopped after 20 minutes by addition of the stop solution. The optical density was determined spectrophotometrically at 450 nm. All samples and standards were run in duplicate and data analysis was performed using Molecular Devices Softmax Pro software (version 2.2.1).

II. Results and discussion

A. Mortality and clinical observations

There were no unscheduled deaths during the study. There were no clinical signs for any of the study animals after the start of treatment.

B. Water and food consumption and dietary intake

Water consumption was statistically significantly less than vehicle control ($p < 0.05$) at weeks 2 and 3 for the 1000 ppm fluoxastrobin group and at weeks 3 and 4 for the 8000 ppm fluoxastrobin group. These changes (0.81-0.84 × vehicle control) are considered related to fluoxastrobin treatment.

Food consumption for the fluoxastrobin treated rats was statistically significantly reduced ($p < 0.05$) from vehicle control only at week 1 for the 8000 ppm group. The toxicological significance of this change is unclear because thereafter there were no statistically significant changes in food consumption.

Cyclophosphamide Group 5 mean water consumption values were statistically significantly reduced ($p < 0.05$) compared to vehicle control for weeks 2, 3, and 4. However, the changes observed for weeks 2 and 3 are not considered toxicologically relevant because cyclophosphamide dosing did not begin until week 4. Food consumption was statistically significantly less than vehicle control for week 4, which in addition to the loss of body weight, correlates to the period of cyclophosphamide dosing.

Table 5.8.2- 1: Mean weekly water consumption (g/rat)

	Week 1			Week 2			Week 3			Week 4		
Group	Mean	SE	SD	Mean	SE	SD	Mean	SE	SD	Mean	SE	SD
Vehicle Control	269.9	17.6	55.7	326.9	22.4	70.8	357.0	29.9	94.6	348.5	25.7	81.3
Fluoxastrobin 125 ppm	255.9	10.6	33.5	291.4	12.9	40.8	323.3	14.9	47.1	337.7	12.0	37.9
Fluoxastrobin 1000 ppm	222.9	10.6	33.5	267.9*	13.3	42.1	301.4*	18.5	58.5	307.5	16.4	51.9
Fluoxastrobin 8000 ppm	226.1	16.8	53.1	278.5	21.9	69.3	289.3*	22.6	71.5	285.8*	22.2	70.2
Positive Control	233.6	11.0	34.8	264.2*	13.3	42.1	276.1*	14.9	47.1	289.6*	14.0	44.3

SE = standard error, SD = standard deviation (calculated as SD = SE* √ n)
* Significantly different from vehicle control ($p < 0.05$)

Table 5.8.2- 2: Mean weekly food consumption (g/rat)

	Week 1			Week 2			Week 3			Week 4		
Group	Mean	SE	SD	Mean	SE	SD	Mean	SE	SD	Mean	SE	SD
Vehicle Control	165.1	2.7	8.5	185.9	4.3	13.6	193.9	5.3	16.8	196.6	5.1	16.1
Fluoxastrobin 125 ppm	166.3	3.1	9.8	184.6	4.4	13.9	195.6	5.8	18.3	199.7	5.2	16.4
Fluoxastrobin 1000 ppm	163.0	3.4	10.8	187.4	5.0	15.8	197.7	5.4	17.1	198.4	5.5	17.4
Fluoxastrobin 8000 ppm	146.3*	3.1	9.8	174.8	2.7	8.5	184.3	2.7	8.5	191.2	4.1	13.0
Positive Control	159.2	3.4	10.8	178.9	4.2	13.3	186.0	4.9	15.5	155.3*	4.5	14.2

C. Body weight

Weekly body weights for the 8000 ppm group were statistically significantly reduced ($p < 0.05$) on Days 8, 15, and 22 (~0.93 × vehicle control) compared to vehicle control, but comparable to vehicle control at termination (Day 29). The weekly decreases for the 8000 ppm group are considered of marginal toxicological significance because the decreases were not > 10%, and the terminal body weight was comparable to vehicle control. One control animal lost 60 g of body weight the week prior to scheduled euthanasia, but there were no observations that would provide an explanation for this loss.

Cyclophosphamide mean terminal body weight was statistically significantly reduced ($p < 0.05$) compared to vehicle control. This change correlates with a decrease in week 4 mean food consumption that occurred during the 6-day period of cyclophosphamide dosing.

Table 5.8.2- 3: Terminal body weight (g)

Group	Mean	SE	SD
Vehicle Control	416.6	12.05	38.11
Fluoxastrobin 125 ppm	416.6	6.70	21.19
Fluoxastrobin 1000 ppm	423.8	6.62	20.93
Fluoxastrobin 8000 ppm	405.9	5.44	17.20
Positive Control	382.4*	8.46	26.75

SE = standard error, SD = standard deviation (calculated as SD = SE × √ n)
* Significantly different from vehicle control ($p < 0.05$)

D. Organ weights

There were no fluoxastrobin-related effects on mean absolute and relative spleen weights (per 100 g body weight).

Cyclophosphamide treated rats had absolute and relative spleen weights significantly less than vehicle control (0.46 × and 0.50 × vehicle control, respectively).

There were no fluoxastrobin-related effects on mean absolute and relative thymus weights (per 100 g body weight).

Cyclophosphamide treated rats had absolute and relative thymus weights significantly less than vehicle control (0.26 × and 0.29 × vehicle control, respectively).

Table 5.8.2- 4: Spleen weight

	Absolute spleen weight (g)			Relative spleen weight (g/kg 100 g bw)		
Group	Mean	SE	SD	Mean	SE	SD
Vehicle Control	0.890	0.028	0.089	0.215	0.009	0.0285
Fluoxastrobin 125 ppm	0.815	0.033	0.104	0.195	0.006	0.0190
Fluoxastrobin 1000 ppm	0.812	0.022	0.070	0.192	0.005	0.0158
Fluoxastrobin 8000 ppm	0.790	0.037	0.117	0.195	0.011	0.0348
Positive Control	0.409	0.022	0.070	0.107	0.005	0.0158

SE = standard error, SD = standard deviation (calculated as SD = SE × √ n)
* Significantly different from vehicle control ($p < 0.05$)

Table 5.8.2- 5: Thymus weight

Group	Absolute thymus weight (g) Mean	SE	SD	Relative thymus weight (g/kg 100 g bw) Mean	SE	SD
Vehicle Control	0.755	0.020	0.063	0.182	0.004	0.013
Fluoxastrobin 125 ppm	0.692	0.050	0.158	0.165	0.010	0.032
Fluoxastrobin 1000 ppm	0.732	0.054	0.171	0.172	0.012	0.038
Fluoxastrobin 8000 ppm	0.606	0.041	0.130	0.149	0.010	0.032
Positive Control	0.199*	0.015	0.047	0.052*	0.003	0.009

SE = standard error, SD = standard deviation (calculated as SD = SE × √ n)
* Significantly different from vehicle control ($p < 0.05$)

D. Immune response – SRBC-specific IgM response

Anti-SRBC IgM was measured on Day 29, 6 days post-intravenous immunization with 1×10^7 SRBC/rat (0.2 mL of 5×10^7 SRBC/mL) concentration. This immunization concentration produced a good antibody response and significant immunosuppression with the cyclophosphamide immunomodulatory positive control (IgM concentration = 0.12 × vehicle control).

There appeared to be a trend of a dose-related decrease in anti-SRBC IgM with increasing dose of fluoxastrobin. However, this change was not statistically significant.

Table 5.8.2- 6: Anti-SRBC IgM (U/mL)

Group	Mean	SE	SD
Vehicle Control	4255.1	884.8	2797.98
Fluoxastrobin 125 ppm[a]	4376.7	1170.17	3510.51
Fluoxastrobin 1000 ppm	3999.5	1151.5	3641.36
Fluoxastrobin 8000 ppm	3160.4	738.85	2336.45
Positive Control	514.2*	78.41	247.95

SE = standard error, SD = standard deviation (calculated as SD = SE × √ n)
* Significantly different from vehicle control ($p < 0.05$); [a] one value with an anti-SRBC IgM of 20707.9 U/mL was excluded from the calculations because it was regarded as an outlier

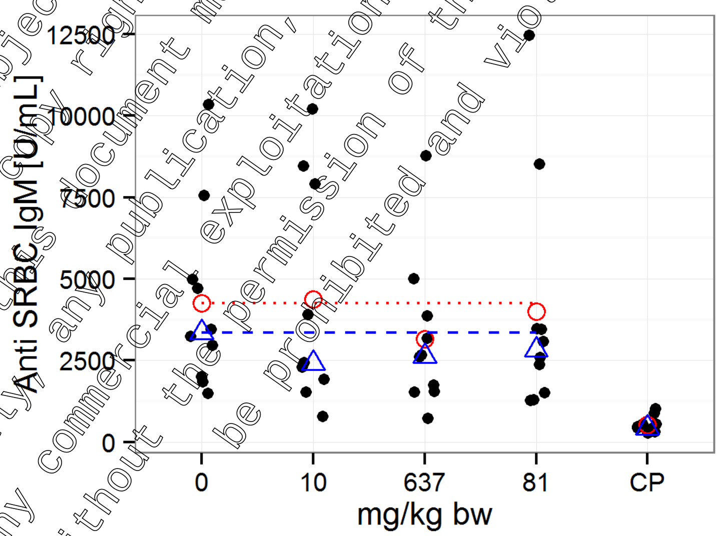

Figure 5.8.2- 1: Individual animal response against approximate fluoxastrobin dose with means (red circles) and medians (blue triangles). Superimposed lines additionally show negative control mean (dotted) and median (dashed) for reference. (CP = 15 mg/kg bw cyclophosphamide) One value with an anti-SRBC IgM of 20707.9 U/mL was excluded from the calculations because it was regarded as an outlier.

III. Conclusion

Based on the conditions of this study the NOEL is 125 ppm (approx. 10 mg/kg bw) based on decreased water consumption at 1000 and/or 8000 ppm (approx. 81 and 637 mg/kg bw) and decreased body weights at 8000 ppm (approx. 637 mg/kg bw).

Based on the immunotoxicity indices of this study the fluoxastrobin NOAEL for immunotoxicity is 8,000 ppm (approx. 637 mg/kg bw).

Supplementary studies on impurities of fluoxastrobin

All necessary studies were presented and evaluated during the EU process for Annex I listing. Please refer to the DAR and the baseline dossier of fluoxastrobin.

In addition, toxicological studies conducted with HEC 5725-E-CL-PMD are also considered supportive to justify the limits of specified impurities.

Report:	KCA 5.8.2/20[redacted]; 2004; M-107900-01-1
Title:	HEC 5725-E-CL-PMD - V79/HPRT-Test in vitro for the detection of induced forward mutations
Report No.:	AT00945
Document No.:	M-107900-01-1
Guideline(s):	Directive 2000/32/EC, method B.17.; OECD 476, US-EPA 712-C-98-221, OPPTS 870.5300
Guideline deviation(s):	None
GLP/GEP:	**Yes**

I. Materials and methods

A. Materials

1. Test material:

Name:	HEC 5725-E-CL-PMD
CAS number:	193741-62-7
Description:	White powder
Lot/Batch no:	BID 4014-028
Purity:	99.7% (w/w)
Stability of test compound:	guaranteed for study duration; a stability test in the solvent did not reveal significant degradation of the active ingredient

2. Vehicle and controls:

Vehicle:	DMSO, 1% final concentration
Vehicle control:	medium with vehicle with or without metabolic activation
Negative control:	Medium
Positive controls:	900 µg/ml EMS without metabolic activation 20 µg/ml DMBA with metabolic activation

3. Test system:

Cell line:	Chinese hamster V79 cells
Source:	[redacted] Germany
Culture conditions:	V79 cell stocks stored in liquid nitrogen. Laboratory cultures maintained in plastic tissue culture vessels at 37°C in a humidified atmosphere containing approximately 5% CO_2. Exponential growth of cell cultures maintained by subculturing at least twice a week. The cells were checked for karyotype stability and mycoplasma contamination. To keep the number of spontaneous 6-TG resistant mutants at a low level, cell

cultures were subcloned by plating about 1000 cells per culture vessel at least every two weeks. If necessary, the spontaneous frequency of HPRT-mutants was additionally reduced by supplementing the culture medium with thymidine (9 µg/mL), hypoxanthine (10 µg/ml), glycine (22.5 µg/mL) and methotrexate (0.3 µg/mL).

Medium: Hypoxanthine-free Eagle's Minimal Essential Medium (MEM) supplemented with L-glutamine (2 mM), MEM-vitamins, NaHCO3, penicillin (100 units/mL), streptomycin (100 µg/mL) and heat-inactivated fetal calf serum (final concentration: 10%) (Seromed). During treatment with the test item, the serum content was reduced to 2%. For selection of mutants, a hypoxanthine-free culture medium was used, containing 10 ug/mL of 6-thioguanine (6-TG).

Metabolic activation: S9 mix was prepared from the livers of Aroclor 1254 induced male Sprague Dawley rats, protein concentration: 26.4 mg/mL.

B. Study design and methods

1. Treatment:

Concentrations:

Exposure period	S9 mix	Test item concentrations [µg/mL]
Cytotoxicity		
5 h	−	0.05, 0.1, 1, 5, 10, 20, 40[P], 80[P]
5 h	+	0.05, 0.1, 1, 5, 10, 20, 40, 80
Mutagenesis		
24 h	−	5, 10, 20, 40[P], 60[P], 80[P]
24 h	+	5, 10, 20, 40, 60[P], 80[P]

[P] : Precipitation visible to the unaided eye

For each test solution or control two parallel cultures were used.

Incubation conditions: 5 or 24 hours, at 37°C in a humidified atmosphere with 5% CO_2

2. Statistical analysis:

Mutant frequencies submitted to a weighted analysis of variance as well as to a weighted recursive regression, both with Poisson derived weights. Mutant frequencies based on less than 5 plates and/or on a relative survival to treatment and/or a relative population growth and/or an absolute cloning efficiency below 10% are not included in the statistical analysis. The two mutant frequency values obtained per group are, although somewhat related, considered as independent measurements thus increasing the power of the statistical tests applied. Separate analyses will be run for each trial in order to examine the consistency of the results. All acceptable groups are included in the weighted analysis of variance followed by pair-wise comparisons to the vehicle control on a nominal significance level of alpha = 0.05 using the Dunnett test. The regression analysis part is performed on the basis of the actual concentrations thereby omitting the positive, negative and vehicle controls. If there is a significant concentration related increase of the mutant frequency (alpha = 0.05) in the main analysis the highest concentration will be dropped and the analysis will be repeated. This procedure will be repeated until $p > 0.05$. In that way eliminated concentrations are flagged correspondingly.

II. Results and discussion

A. General Remarks

In the absence and in the presence of S9 mix Chinese hamster V79 cells were exposed to HEC 5725-E-CL-PMD at concentrations of up to and including 80 µg/ml.

Without S9 mix substance precipitation occurred in the medium at the concentration 40 µg/ml and above. With S9 mix precipitation was noted at the concentration 60 µg/ml and above. Based on these findings, at least two precipitating concentrations were tested in each trial.

The means of the absolute cloning efficiency for the vehicle controls in the mutation experiments were 60.0% to 79.2% in the experiments without activation. In experiments with metabolic activation 50.0% to 78.5% were observed. These results demonstrate good cloning conditions for the experiments.

B. Mutation Assay

Without metabolic activation

Under nonactivation conditions two trials were performed. The mutant frequencies of the negative controls and of the vehicle controls were all within the normal range. The positive control EMS induced clear mutagenic and statistically significant effects in all trials.

For HEC 5725-E-CL-PMD treated cultures concentration-related decreases were observed in relative population growth. Relevant HEC 5725-E-CL-PMD induced increases in mutant frequencies could not be found. In addition, the overall statistical analysis reveals no statistically significant increases.

Therefore, HEC 5725-E-CL-PMD was evaluated as non-mutagenic in the non-activation trial.

Table 5.8.2/20- 1 Summary of results without metabolic activation

	Concentration [µg/mL]	S9 mix	Growth rel to vehicle control (%)	Mutant colonies per 10^6 cells	Growth rel to vehicle control (%)	Mutant colonies per 10^6 cells
24 h treatment			Culture I		Culture II	
Negative Control	-	–	83.9	0.7	85.5	0.5
	-	–	104.3	1.9	89.6	0.9
Vehicle Control	-	–	100.0	0.8	100.0	2.0
	-	–	100.0	0.7	100.0	1.1
EMS	900	–	48.6	321.6	58.5	882.8
	900	–	57.3	500.8	35.8	668.0
HEC 5725-E-CL-PMD	5	–	115.7	1.9	78.6	4.8
	5	–	72	1.8	40.7	0.7
	10	–	84.7	0.8	49.4	4.3
	10	–	68.0	0.6	26.4	0.3
	20	–	87.2	0.6	34.2	2.9
	20	–	53.0	0.5	28.6	0.7
	40 P/-	–	82.0	1.4	29.8	0.6
	40 P/P	–	64.6	0.8	18.5	0.6
	60 P/P	–	63.0	0.9	18.8	5.0
	60 P/P	–	46.2	6.5	15.9	0.8
	80 P/P	–	35.9	1.0	16.1	3.5
	80 P/P	–	29.5	2.0	11.2	0.9

P: Precipitation Culture 1-Culture 2

With metabolic activation

Two trials were performed with S9 mix. In all experiments, cytotoxic effects were induced. The mutant frequencies of the negative controls and of the vehicle controls were all within the normal range. The positive control DMBA induced a clear mutagenic and statistically significant effect in all trials. The HEC 5725-E-CL-PMD treated cultures showed concentration-related decreases in relative population growth.

HEC 5725-E-CL-PMD induced no relevant increases in mutant frequencies. In addition, the overall statistical analysis reveals no statistically significant increase.

With metabolic activation HEC 5725-E-CL-PMD was therefore evaluated as non-mutagenic.

Table 5.8.2/20- 2 Summary of results with metabolic activation

	Concentration [µg/mL]	S9 mix	Growth rel to vehicle control (%)	Mutant colonies per 10^6 cells	Growth rel to vehicle control (%)	Mutant colonies per 10^6 cells
24 h treatment			Culture I		Culture II	
Negative Control	-	+	59.5	2.5	79.5	2.1
	-	+	71.1	2.4	83.2	3.0
Vehicle Control	-	+	100.0	1.0	78.5	0.5
	-	+	100.0	1.7	70.8	2.9
DMBA	20	+	39.3	89.7	72.7	139.6
	20	+	45.2	154.2	63.8	108.4
HEC 5725-E-CL-PMD	5	+	64.1	0.8	60.2	5.5
	5	+	78.4	1.5	69.0	1.8
	10	+	-	-	87.3	1.0
	10	+	-	-	85.8	5.7
	20	+	59.3	0.5	71.0	6.5
	20	+	55.7	0.9	64.5	3.2
	40	+	75.5	2.3	89.0	1.9
	40	+	61.1	0.8	52.3	1.8
	60 P/P	+	63.1	1.4	51.8	1.6
	60 P/P	+	66.0	0.5	60.8	1.4
	80 P/P	+	60.0	0.9	65.2	2.6
	80 P/P	+	53.3	0.5	63.2	0.7

P: Precipitation Culture 1-Culture 2

III. Conclusions

The test item HEC 5725-E-CL-PMD did not induce gene mutations at the HPRT locus in V79 cells under the experimental conditions reported. Therefore, HEC 5725-E-CL-PMD is considered to be non-mutagenic in this HPRT assay.

Report:	KCA 5.8.2/21 [redacted],; 2004; M-002507-01-1
Title:	HEC 5725-E-CL-PMD - In vitro chromosome aberration test with chinese hamster V79 cells
Report No.:	AT01110
Document No.:	M-002507-01-1
Guideline(s):	Directive 2000/32/EC, B.10; OECD 473; US-EPA 712-C-98-223, OPPTS 870.5375
Guideline deviation(s):	None
GLP/GEP:	**Yes**

I. Materials and methods

A. Materials

1. Test material:

Name:	HEC 5725-E-CL-PMD
CAS number:	193741-62-7
Description:	white coarse-grained powder
Lot/Batch no:	BID 4014-028
Purity:	99.7%
Stability of test compound:	guaranteed for study duration; HEC 5725-E-CL-PMD is stable in the vehicle at room temperature at concentrations ranging from 0.01 mg/mL to 50 mg/mL for at least four hours

2. Vehicle and controls:

Vehicle:	DMSO
Positive control:	Mitomycin C in Hanks' balanced salt solution, final dilution in the medium 0.1 µg/mL (4 hours) and 0.03 µg/mL (18 hours) Cyclophosphamide in Hanks' balanced salt solution, final dilution in medium 2 µg/mL

3. Test system:

Cell line:	Chinese hamster V79 cells
Source:	[redacted]
Culture conditions:	Chinese hamster V79 cells stored in liquid nitrogen, were normally grown in 20 mL medium and 75 cm^2 flasks or under comparable conditions. Incubation of the cells was always performed at 37°C in a CO_2-incubator (5% CO2). Cells were grown in medium containing 10% fetal calf serum.
Medium:	Eagle's Minimal Essential Medium (MEM) supplemented with L-glutamine (2 mM), MEM-vitamins, 0.225% NaHCO3, penicillin (50 units/mL), streptomycin (50 µg/mL) and heat-inactivated fetal calf serum (final concentration: 10%).
Metabolic activation:	S9 mix was prepared from the livers of Aroclor 1254 induced male Sprague Dawley rats, protein concentration: 26.2 mg/mL

B. Study design and methods

1. Treatment

Concentration:

Test item	S9 mix	Test item concentrations [µg/mL]	Harvest time
4 h treatment			
Test item	−/+	0, 20, 40, 80	18 h
Mitomycin C	−	0.1	
Cyclophosphamide	+	2.0	
Test item	−/+	0, 80	30 h
18 h treatment			
Test item	−	0, 3, 6, 9, 12, 15	18 h
Mitomycin C	−	0.03	

Incubation conditions: At 37°C in a CO_2-incubator (5% CO_2)

2. Statistical analysis:

Statistical analysis performed by pair-wise comparison of test item-treated and positive control groups to the respective solvent control group.
Mitotic index statistically analyzed (provided that it was reduced compared to the mean of the corresponding solvent control) using the one-sided chi2-test.
Numbers of metaphases with aberrations (including and excluding gaps) and of metaphases with exchanges were compared (provided that these data superceded the respective solvent control). The statistical analysis followed the recommendations outlined by [redacted] et al. (1989). Fisher's exact test was used for the statistical evaluation.
A difference was considered to be significant, if the probability of error was below 5%.

II. Results and discussion

A. General Remarks

Without and with S9 mix substance precipitation in the medium started microscopically at 20 µg/mL and macroscopically at 40 ug/ml.

B. Mitotic Index

Without metabolic activation

In comparison to the solvent control, the mitotic indices in the treated cultures were relevantly reduced at 80 µg/ml (4 hours treatment) and at 3 µg/ml and above (18 hours treatment). The cultures treated with mitomycin C showed no reduction in mitosis rate.

With metabolic activation

In comparison to the solvent control, the treated cultures showed a relevant reduction of the mitosis rate at 80 ug/ml. The positive control cyclophosphamide also reduced the mitosis rate.

C. Survival Index

Without metabolic activation

In comparison to the solvent control, the survival indices in the treated cultures were relevantly reduced at 80 ug/ml (4 hours treatment) and at 9 ug/ml and above (18 hours treatment). The cultures treated with mitomycin C showed only for the 4 hours treatment a reduction in survival rate.

With metabolic activation

In comparison to the solvent control, the treated cultures showed a relevant reduction of the survival rate at 80 ug/ml. The positive control cyclophosphamide also reduced the survival rate.

D. Chromosome aberrations

Based on the results of the survival index and of the mitotic index, the following concentrations of the 18 hours treatments were selected for reading: 3, 6, 9 µg/mL.

Without metabolic activation

No biologically relevant and statistically significant increases of numbers of metaphases with aberrations were detected after 4 hours treatment and total culture times of 18 or 30 hours. The same was true for a treatment period and total culture time of 18 hours.

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

With metabolic activation

No biologically relevant and statistically significant increases of metaphases with aberrations were detected after total culture times of 18 or 30 hours.

The positive control cyclophosphamide induced statistically significant and biologically relevant increases of metaphases with aberrations and demonstrated the sensitivity of the test system and the activity of the used S9 mix.

Table 5.8.2/21- 1 Summary of cells with structural aberrations

Test item	Concentration (µg/mL)	+/- S9	Cells scored	Cells with aberrations (%)			Mitotic Index (mean %)
				Including gaps	Excluding gaps	Exchanges	
4 hour treatment, harvest time 18 h							
DMSO	0	–	200	4.0	3.5	1.0	100
HEC 5725-E-CL-PMD	20	–	200	2.0	2.0	1.5	87.1
	40	–	200	3.0	3.0	0.0	97.5
	80	–	200	3.5	3.0	1.0	84.7*
Mitomycin C	0.1	–	200	53.5**	52.0**	28.0**	103.2
DMSO	0	+	200	3.5	3.5	1.5	100
HEC 5725-E-CL-PMD	20	+	200	3.5	3.0	1.0	120.6
	40	+	200	2.0	1.5	0.0	118.6
	80	+	200	4.0	4.0	0.0	108.3
Cyclophosphamide	2	+	200	55.5**	55.0**	26.5**	59.8**
4 hour treatment, harvest time 30 h							
DMSO	0	–	200	2.5	2.0	1.0	100.0
HEC 5725-E-CL-PMD	80	–	200	3.0	2.5	0.5	57.9**
DMSO	0	+	200	2.0	2.0	0.5	100.0
HEC 5725-E-CL-PMD	80	+	200	3.0	2.5	1.0	77.2**
18 hour treatment, harvest time 18 h							
DMSO	0		200	1.0	0.5	0.0	100
HEC 5725-E-CL-PMD	3		200	1.0	1.0	0.0	72.3*
	6	–	200	2.0	2.0	0.5	50.9**
	9		200	1.5	1.5	0.0	43.8**
Mitomycin C	0.3	–	200	34.5**	34.0**	11.5**	111.6

* statistical significance $p < 0.05$ ** statistical significance $p < 0.01$

III. Conclusion

HEC 5725-E-CL-PMD did not induce chromosome aberrations in Chinese hamster V79 cells when tested up to 80 µg/mL in either the absence or the presence of a rat liver metabolic activation system (S9). Based on the results of this test, HEC 5725-E-CL-PMD is considered not to be clastogenic for mammalian cells in vitro.

CA 5.8.3 Endocrine disrupting properties

It should be noted that to date no clear criteria are available in the EU to define endocrine disrupting properties. Furthermore, the toxicological profile of fluoxastrobin does not meet the EU interim criteria for endocrine disrupting properties.

Fluoxastrobin caused no tumors in rats and mice which were assessed to be treatment-related and caused no toxicological relevant findings in endocrine tissues observed in the apical toxicological studies with fluoxastrobin as assessed already for Annex I inclusion.

Minor effects in endocrine tissues occurred as follows:

Regarding thyroid-related changes, in the 90-day dog study T3 values were transiently decreased in female dogs at the mid- and high dose in the absence of thyroid weight changes and histopathological findings. The lower T3 values are considered to be secondary due to liver enzyme induction (increased UDP-GT in the mid- and high-dose females) and thyroid hormone clearance. In the 1-year dog study T3 values were not affected. The increase in relative thyroid weight at all dose groups was not considered toxicologically relevant in the absence of any substance-related pathological findings in the thyroid. Thus, without an adverse effect on the thyroid itself, no indication for a direct endocrine activity of fluoxastrobin can be assumed.

Findings on male reproductive tissues were observed in one of the 28-day toxicity studies in rats and were considered to be secondary to reduced body weight gain (by 15-30%). Histopathological changes of these tissues were neither observed in the 2-generation study nor in other short-term studies in rats. In the 2-generation study in rats, a slight delay in preputial separation was observed in pups secondary to the reduced pup growth at the top dose. This pattern is clearly distinct from what would be expected for an endocrine-mediated effect; a primary endocrine effect would delay in preputial separation in the presence of higher body weight at the day of preputial separation, due to continuous growth of the pup over time. Furthermore, no effect on the anogenital distance was observed in F2 pups of the 2-generation study with fluoxastrobin.

During the previous EU review the possible influence of fluoxastrobin on the female endocrine system was already discussed due to the higher incidence of uterine adenocarcinoma observed in the 2-year chronic/carcinogenicity study in rats. The origin of these lesions was demonstrated to be spontaneous and thus unrelated to treatment with fluoxastrobin ([redacted].,; 2004; M-082214-01-1; as requested by RMS UK in their letter COP 2016/00206, Ref. W001721642, BCS response is updated in new document M-549514-01-1). This was confirmed by an expert meeting EPCO 14 (11.-14.10.2004): "*The meeting agreed that the historical control data and particularly data from a study run concurrently suggested the finding of uterine adenocarcinoma was incidental and that the concurrent control was low.*"

Overall, it can be concluded that, based on a complete toxicological data set, there is no evidence for direct endocrine activity of fluoxastrobin.

CA 5.9 Medical data

CA 5.9.1 Medical surveillance on manufacturing plant personnel and monitoring studies

Details on medical surveillance on manufacturing plant personnel and monitoring studies are provided in Document JCA, Confidential Information.

Report:	KCA 5.9.1/02 [redacted] W; 2015; M-520339-01-1
Title:	Summary of medical data known for fluoxastrobin
Report No.:	M-520339-01-1
Document No.:	M-520339-01-1
Guideline(s):	not applicable
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

Report:	KCA 5.9.1/03 [redacted] 2015; M-532647-01-1
Title:	Summary of medical data known for Fluoxastrobin provided to Bayer CropScience AG
Report No.:	M-532647-01-1
Document No.:	M-532647-01-1
Guideline(s):	EU Regulation 1107/2009; EU Regulation 283/2013
Guideline deviation(s):	not applicable
GLP/GEP:	**no**

CA 5.9.2 Data collected on humans

No cases of human poisoning have been reported up to now.

CA 5.9.3 Direct observations

Up to now there are no direct observations available.

CA 5.9.4 Epidemiological studies

Up to now there are no epidemiological studies available.

CA 5.9.5 Diagnosis of poisoning (determination of active substance, metabolites), specific signs of poisoning, clinical tests

No human cases have been reported; in animal experiments no specific symptoms have been seen.

CA 5.9.6 Proposed treatment: first aid measures, antidotes, medical treatment

First Aid:

- Remove patient from exposure/terminate exposure
- Thorough skin decontamination with copious amounts water and soap, if available with polyethylenglykol 300 followed by water. Note: Most formulations with this active ingredient can be decontaminated with water (and soap), so for formulations polyethylenglykol 300 is not required.
- Flushing of the eyes with lukewarm water for 15 minutes
- Induction of vomiting does not seem to be required. It should only be considered if a large amount has been swallowed, if the ingestion was less than one hour ago, and if the patient is fully conscious.
  NOTE: Induction of vomiting is forbidden, if a formulation containing organic solvents has been ingested!

Treatment:

- Gastric lavage does not seem to be required in regard of the low toxicity of the compound
- The application of activated charcoal and sodium sulphate (or other cathartic) might be considered in significant ingestions.
- As there is no antidote, treatment has to be symptomatic and supportive.

CA 5.9.7 Expected effects of poisoning

No persisting effect of poisoning has to be expected.

Appendix 1 - Proposed toxicological classification of fluoxastrobin

This appendix provides a detailed comparison of potentially classification-relevant toxicological findings of fluoxastrobin with the respective applicable CLP criteria (following the Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, June 2015). As an outcome of this exercise, proposals for classification / non-classification are made for acute toxicity, skin irritation, eye irritation, skin sensitization, germ cell mutagenicity, carcinogenicity, reproductive toxicity, STOT-SE, STOR-RE.

It has to be noted, that fluoxastrobin was already assessed by the Technical Committee on Classification and Labelling in Arona, 15-16 May 2007, with the final recommendations for classification and labelling to be forwarded to ECHA.

QUOTE

P637 (UK) **Fluoxastrobin** **CAS : 193740-76-0** AND **Fluoxastrobin (ISO)** **CAS: 361377-29-9** **Classification:** **No classification for health effects** ***Agreed 0507*** **Labelling:** None	The C&L proposal for fluoxastrobin was received from UK in document ECBI/28/07. All MS experts agreed at the *May 2007* meeting not to classify fluoxastrobin for health effects. In addition, the same conclusion was drawn for Fluoxastrobin ISO with CAS number 361377-29-9 **→ Next ATP, but only if classified for ENV effects.**

UNQUOTE

ACUTE TOXICITY, SKIN IRRITATION, EYE IRRITATION, SKIN SENSITISATION

According to the ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, June 2015, the results of the acute toxicological studies

- oral LD_{50} rat >2000 mg/kg bw (M-012717-01-1, M-012735-01-1)
- dermal LD_{50} rat >2000 mg/kg bw (M-012730-01-1)
- inhalation LC_{50} rat >5 mg/L (M-008820-01-1)
- no skin irritation (M-012662-02-1)
- slight eye irritation which does not warrant classification (M-012669-02-1)
- no skin sensitization (M-012720-01-1, M-105571-01-1, M-278315-01-1)

do **not trigger any respective classification**. Furthermore, fluoxastrobin does not show a phototoxic potential (M-497574-01-1), see also Table 5.2-1.

GERM CELL MUTAGENICITY

According to the above mentioned ECHA Guidance a classification for germ cell mutagenicity Category 2 is based on:

A) Positive somatic cell mutagenicity tests in vivo, in mammals; or
B) Other positive in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays; or
C) Positive in vitro mammalian mutagenicity assays for substances which also show chemical structure activity relationship to known germ cell mutagens.

A summary of available mutagenicity studies conducted with fluoxastrobin is provided in the following table (see also Table 5.4-1):

Study	Result		Reference
	+S9	-S9	
Bacterial point mutation assay (Ames test) in *S. typhimurium* strains	Negative	Negative	M-012700-01-1
Bacterial point mutation assay (Ames test) in *S. typhimurium* strains	Negative	Negative	M-012732-01-1
Bacterial point mutation assay (Ames test) in *S. typhimurium* strains	Negative	Negative	M-278050-01-1
Clastogenicity *in vitro* (V79 CHL cells)	Negative	Negative	M-012703-01-1
Mammalian cell mutation assay (V79 CHL cells – HPRT locus)	Negative	Negative	M-012722-01-1
Mammalian cell mutation assay (V79 CHL cells – HPRT locus)	Negative	Negative	M-078586-01-1
Micronucleus assay (*In vivo* mouse bone marrow)	Negative (Clear evidence of systemic toxicity for fluoxastrobin and/or its metabolites reaching the bone marrow)		M-012747-01-1

Based on these results, the aforementioned classification criteria A) and B) are not met. Classification criterion C) is also not met since fluoxastrobin does not show a chemical structure activity relationship to known germ cell mutagens.

Based on these data it is concluded that **no classification for germ cell mutagenicity** is applicable for fluoxastrobin according to the above mentioned ECHA Guidance.

CARCINOGENICITY

According to the above mentioned ECHA Guidance a classification for carcinogenicity requires an increased incidence of neoplasms due to exposure to the substance.

In the two year rat study, besides body weight effects, local effects in the intestine and an increased number of mast cells, altered calcium/phosphate homeostasis and decreased calcium content of bone were observed. However, there were no clear substance related pathological effects on the kidney or urinary bladder of rats.

As agreed by the experts' meeting, the higher incidence of uterine adenocarcinoma compared to concurrent controls occurred were considered of spontaneous origin and thus unrelated to treatment with fluoxastrobin (M-082214-01-1; as requested by RMS UK in their letter COM 2016/00206, Ref. W001721642, BCS response is updated in new document M-549514-01-1, see document MCA 5.5):

- The incidence of adenocarcinoma at the top dose (20%) was similar to the incidence (24%) reported for a control group of an almost parallel running study (same rat strain, same breeder, same laboratory).
- Occurrence (beyond week 80 with one exception) of these tumours was similar in high dose and study controls and also similar in controls of the concurrent study.
- The incidence of focal and diffuse glandular hyperplasia at the top dose (12%) was lower than the incidence (22%) of glandular cystic hyperplasia in controls of the concurrent study (lesions are comparable).
- There were no significant effects on reproductive performance in the multigeneration study with fluoxastrobin (indicating that the uterine adenocarcinoma are not endocrine mediated).

Furthermore, the overall incidence of tumour-bearing animals, the time of occurrence and the pattern of neoplastic findings did not indicate a carcinogenic effect.

In a 18 month mouse study, there was no increase in neoplastic findings, also the time of occurrence and the pattern of neoplastic findings in all organs and tissues did not indicate a treatment-related effect.

Hence, it is concluded that fluoxastrobin is not carcinogenic in rats or mice and that **classification for carcinogenicity is not warranted**.

REPRODUCTIVE TOXICITY

As detailed in the following tables for the respective individual animal studies, fluoxastrobin caused only non-specific developmental toxicity secondary to very strong maternal toxicity; this does not warrant any reproductive toxicity classification.

In the 2-generation study, adverse developmental effects, ie reduced body weight gain, delayed development (e.g. time to preputial separation) and reduced weight of thymus and spleen of pups were seen at the top dose. NOAEL for reproduction is 10000 ppm (742-764 mg/kg bw/day) and the parental NOAEL is 1000 ppm (74-87 mg/kg bw/day) based on reduced body weight gain and reduced thymus weight in females at 10000 ppm (764-871 mg/kg bw/day). The NOAEL for developmental effects in the rat multigeneration study is 1000 ppm (171 mg/kg bw/day) based on reduced body weight gain, delayed development (delay in preputial separation), reduced thymus and spleen weight observed at 10000 ppm (1625 mg/kg bw/day).

2-generation study in rats (diet), 0, 100, 1000, 10000 ppm, (M-088589-02-1)	
Parental effects:	NOAEL: 1000 ppm (74-87 mg/kg bw/d) Effects at LOAEL 10000 ppm (764-871 mg/kg bw/dr: Reduced body weight gains, increased food consumption, increased liver weight, and decreased thymus weight in females at 10000 ppm,.
Reproductive effects:	NOAEL: 10000 ppm (764 (males) 742 (females) mg/kg bw/d) No adverse effects on reproductive outcome.
Offspring effects:	NOAEL: 1000 ppm (171 mg/kg bw/d in lactating dams) Effects at LOAEL 10000 ppm (1625 mg/kg bw/d): Reduced pup weight gain, decreased pup spleen and thymus weights (no histopathological findings) and slightly delayed preputial separation was observed in pups secondary to the reduced pup growth at this dose. No effect on the anogenital distance was observed in the F2 pups. LDAR: A statistically significant reduction in mean pup body weight was seen at 10000 ppm from days 7 or 14 to day 21 in both F1 and F2 pups. The overall reduction in mean body weight gain (day 0-day 21) was 25-29%. The reduction from lactation day 7 suggests an effect on the mothering or lactational ability of the dams, or of a direct substance-related effect on the pups via the milk. However, direct consumption of test diet by pups from the end of the first week of lactation can occur (ECETOC 2002) and may have contributed to reduced growth.
Classification proposal:	Parental effects (reduced body weight gains, increased food consumption, increased liver weight) were observed at the top dose of 10000 ppm (764-871 mg/kg bw/d). There were no substance-related effects on reproductive parameter. Offspring effects occurred at the highest tested dose of 10000 ppm (1625 mg/kg bw/d in lactating dams) similar to findings which occurred in parental animals at the same dose. According to the ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, June 2015, this constellation of maternal and reproductive / offspring effects does not warrant any reproductive toxicity classification. The classification criteria for a Category 2 classification (see 3.7.2.2. of the ECHA Guidance) are not met: *"... the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

In the rat developmental toxicity study, there was no substance-related adverse maternal or developmental effect. The reduction in ossification of one digit from both forelimbs of fetuses at 300 and 1000 mg/kg bw/day is not considered to be a substance-related adverse effect. The maternal and developmental NOAEL is 1000 mg/kg bw/day in rats.

Developmental toxicity study in rat (gavage), 0, 100, 300, 1000 mg/kg bw/d, (M-012725-01-1)	
Maternal effects:	NOAEL: 1000 mg/kg bw/d
Developmental effects:	NOAEL: 1000 mg/kg bw/d
Classification proposal:	No treatment-related effects on maternal or litter parameters including external fetal observations. There was no evidence of a teratogenic effect. According to the ECHA Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures, Version 4.1, June 2015, the absence of maternal and developmental effects does not warrant any reproductive toxicity classification.

In the rabbit developmental toxicity study, there was evidence for a slight delay in fetal development (slight dilation of lateral brain ventricles in two foetuses of dam No. 3813) at the top dose in the presence of severe (cold ears, severely decreased feed intakes as well as severe body weight loss of dam No. 3813) maternal toxicity. There was also questionable evidence for a slight substance-related increase in the incidence of a common rib cartilage malformation and equivocal evidence for a slight increase in the incidence of one rib variation. The NOAEL for maternal toxicity in the rabbit teratogenicity study is 25 mg/kg bw/day and the developmental is 100 mg/kg bw/day.

Developmental toxicity study in rabbit (gavage), 0, 25, 100, 400 mg/kg bw/d, (M-017448-01-1)	
Maternal effects:	NOAEL: 25 mg/kg bw/d LOAEL: 100 mg/kg bw/d based on reduced food consumption, slight increased incidence of distinct weight loss Effects at top dose 400 mg/kg bw/d: cold ears, soft feces/diarrhea during the first treatment days, reduced amount of feces, transiently severely reduced food and partly water consumption, more pronounced body weight loss mainly during the first treatment week.
Developmental effects:	NOAEL: 100 mg/kg bw/d Effects at LOAEL: 400 mg/kg bw/d based on a slight delay in fetal development (2 cases of slight dilation of brain ventricles in fetuses of dam 3813) at the top dose in the presence of severe maternal toxicity (cold ears, severely decreased feed intakes as well as severe body weight loss of dam 3813). A slight increase in the incidence of a common rib cartilage malformation (3 of 6 affected foetuses from one litter; litter incidence below that of a control group from a parallel study) and an non-statistically increased incidence of slightly thickened 7th left ribs occurred for which a treatment-related effect is questionable. The overall incidence of foetuses or litters with malformations was unaffected up to and including 400 mg/kg bw/d.
Classification proposal:	No treatment-related effects on maternal or litter parameters including external fetal observations. There was no evidence of a teratogenic effect. The classification criteria for a Category 2 classification (see 3.7.2.2. of the ECHA Guidance) are not met: *"... the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects."*

Overall, it is concluded that fluoxastrobin is not teratogenic and that adverse developmental effects could be a consequence of substance-related parental toxicity. **Classification of fluoxastrobin for reproductive toxicity is not justified**.

SPECIFIC TARGET ORGAN TOXICITY – SINGLE EXPOSURE (STOT-SE)

According to the above mentioned ECHA Guidance a classification in **STOT-SE Category 2** is not applicable, if non-lethal significant and/or severe toxic effects on target tissues/organs are not seen in acute toxicity studies up to the following guidance values:

Oral rat	2000 mg/kg bw
Dermal rat or rabbit	2000 mg/kg bw
Inhalation rat, dust / mist / fume	5 mg/l/4h

Furthermore, the ECHA Guidance specifies criteria that trigger a classification for **STOT-SE Category 3**. These criteria are generally independent from the aforementioned guidance values and include transient target organ effects, focusing on overt narcotic effects and respiratory tract irritation (respiratory tract irritation covers two different effects: 'sensory irritation' and 'local cytotoxic effects'). Specifically, the following examples for findings from single and repeated inhalation toxicity studies are mentioned as possible triggers for a STOT-SE Category 3 classification: clinical signs of toxicity (dyspnoea, rhinitis etc) and histopathology (e.g. hyperemia, edema, minimal inflammation, thickened mucous layer) which are reversible.

The relevant acute toxicity studies conducted with fluoxastrobin (a repeated inhalation study is not available) provide the following LOAELs and toxicological effects at the respective LOAELs:

Study	LOAEL Toxicological effects at LOAEL (Reference)
Acute oral rat	2000 mg/kg bw (highest tested dose) No clinical signs. (M-012717-01-1, M-012735-01-1)
Acute dermal rat	2000 mg/kg bw (highest tested dose) No clinical signs. (M-012730-01-1)
Acute inhalation rat	5 mg/L/4h (highest tested dose) Piloerection, ungroomed hair-coat, bradypnea, laboured breathing, serous nasal discharge, reduced motility, limpness on the day of exposure. Reflex measurements on the first postexposure day showed no abnormal reflexes. All clinical signs had resolved within 4 days postexposure. Mean rectal temperatures immediately after exposure were decreased. Slight but transient weight loss during the first three post-exposure days. At necropsy, there were no treatment-related gross lesions in any of the surviving test animals. The rat that died during the observation period showed nasal cavities and trachea with white viscous content and the intestine with red mucoid content. The change in breathing rate and decreased body temperature were attributed to a non-specific response to sensory irritation from exposure to dust. (M-008820-01-1)
Acute oral neurotoxicity rat	2000 mg/kg bw (highest tested dose) No neurotoxicity or general systemic toxicity observed (M-088080-01-1)

A comparison of these LOAELs and toxicological effects with the aforementioned classification criteria reveals that a **STOT-SE Category 2 classification is not warranted**.

Regarding a possible STOT-SE Category 3 classification for "overt narcotic effects", the observed toxicological findings do not indicate such effects; the reduced motility and limpness (acute inhalation) are seen as mild expressions of a generally affected well-being and not as a neuro-pharmaco-toxicological narcotic effect. Therefore, a respective **STOT-SE Category 3 classification is not warranted**.

Regarding a possible STOT-SE Category 3 classification for respiratory tract irritation (sensory irritation or local cytotoxic effects) the observed laboured breathing, serious nasal discharge and red encrustation around the muzzle/nostrils (all reversible within 3 days of exposure) could indicate respiratory tract irritation. However, at necropsy none of the aforementioned histopathological trigger findings were observed. The change in breathing rate and decreased body temperature are attributed to a non-specific response to sensory irritation from exposure to dust, and, thus, not to a specific irritative potential of fluoxastrobin. Altogether, the observed findings are not seen as convincing evidence for a clear and specific respiratory tract irritation due to fluoxastrobin exposure and should therefore **not trigger a STOT-SE Category 3 classification**.

SPECIFIC TARGET ORGAN TOXICITY – REPEATED EXPOSURE (STOT-RE)

According to the above mentioned ECHA Guidance a classification in STOT-RE Category 2 is not applicable, if significant toxic effects observed in 28-day, 90-day or 12-month repeated-dose studies conducted in experimental animals are not seen up to the following guidance values:

Exposure route species	28-day	90-day	12-month	>12-month
Oral rat	300 mg/kg bw/d	100 mg/kg bw/d	25 mg/kg bw/d	no guidance value provided
Demal rat	600 mg/kg bw/d	200 mg/kg bw/d	no guidance value provided	no guidance value provided

In the repeated-dose studies conducted with fluoxastrobin the liver is the main target organ in all tested species (rats, mice and dogs). However, according to the CLP criteria the effects should clearly indicate functional disturbance of morphological changes which are toxicological relevant.

Histological changes were seen in the urinary system of rats and of dogs at doses above the respective guidance values. Male rats were more sensitive than females to the effects of fluoxastrobin on the liver and urinary tract. Other target organs were adrenals, erythrocytes and thyroid but without consistent finding amongst the different studies. Reduced body weight gain was a key finding in dog studies.

In a 28-day dermal study with fluoxastrobin in rats, neither systemic nor local skin effects of toxicological importance were observed up to the highest dose level tested (1000 mg/kg bw/day).

The available toxicity studies do not show significant or severe toxic effects at dose levels requiring classification as STOT-RE.