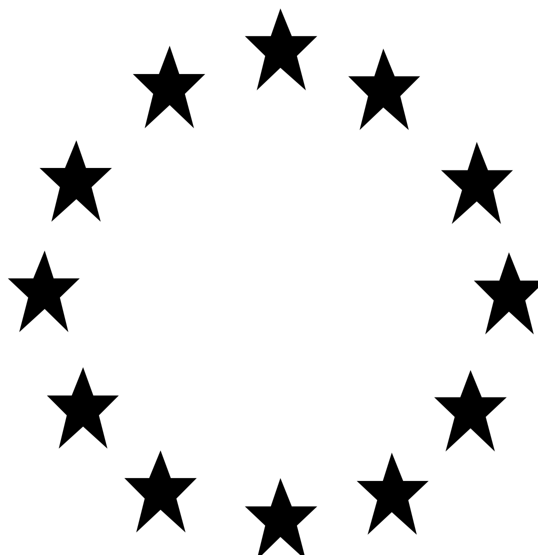

Final addendum to the

Draft Assessment Report (DAR)

- public version -

Initial risk assessment provided by the rapporteur Member State United Kingdom for the new active substance

FLUOXASTROBIN

as referred to in Article 8(1) of Council Directive 91/414/EEC

July 2005

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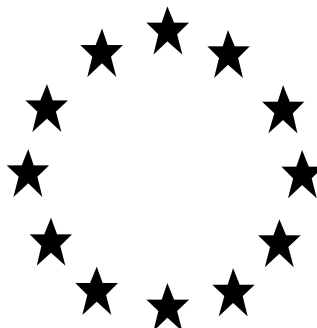

Fluoxastrobin

Addendum 1 to the
Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

Draft: April 2004

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16507/EPCO/BVL/04

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Purpose of this evaluation

Fluoxastrobin (HEC 5725) is a new strobilurin fungicide, developed by Bayer CropScience. The dossier for fluoxastrobin was submitted to the UK on 25 March 2002. The full evaluation of the dossier was presented in the Draft Assessment Report (DAR) on fluoxastrobin, which was submitted to Commission in August 2003. The DAR was distributed to the member states for comment in October 2003. Although the RMS (UK) proposed that fluoxastrobin could be included in Annex I of Directive 91/414/EEC, certain issues were identified that could either delay Annex I inclusion or present an obstacle to product authorisation at the member state level.

1) Many of the toxicological studies had been conducted with high purity batches of fluoxastrobin. Therefore, once a final full production batch of fluoxastrobin was available, a further Ames study (with a representative final full production batch) was to be conducted to provide additional reassurance that low levels of impurities in fluoxastrobin were not of genotoxic concern. The toxicological significance of impurities in fluoxastrobin for skin sensitisation also needed to be addressed. However, the RMS did not consider these data to be essential for the inclusion of fluoxastrobin in Annex I.
2) On the basis of modelling data, it appeared that the soil metabolite M48 could leach to groundwater at levels up to 3.65 micrograms/l. No toxicology studies had been performed on M48 itself. Although M48 is a primary mammalian metabolite and might be expected to be present in studies conducted with parent, in order to address the potential genotoxicity of M48, appropriate studies should be conducted on this metabolite.
3) On the basis of studies conducted with standard species, the risk to aquatic organisms appeared to be acceptable. However, data on the marine mysid shrimp and a range of non-standard fresh water invertebrates indicated that the standard test species were not the most sensitive when exposed to fluoxastrobin. On the basis of these additional data, it appeared that a 15 m buffer zone would be required to mitigate the risk to aquatic species. Such large buffer zones are currently considered to be impracticable by some member states including the UK.

The Notifier has now submitted new data to address the above concerns. In addition, the Notifier:

a) disagreed with the RMS's interpretation of the decreases in the liver enzymes alanine and aspartate aminotransferases, seen in some mammalian toxicology studies,
b) submitted further data on the storage stability of the formulation in commercial packaging and data on the stability of samples taken from supervised crop residue studies when stored deep frozen.

This document presents the evaluation of the data and information presented by the Notifier in response to the above issues. These issues were also the subject of a number of comments submitted by the Notifier, EFSA and the member states on the DAR.

Note: The Notifier has now modified and optimised the production process for fluoxastrobin. Details of the new method of manufacture, revised technical specification and the evaluation of the supporting data on methods of analysis are presented in Fuoxastrobin DAR addendum 2 (Confidential information). Addendum 2 is an extensive revision of volume 4 to the original DAR and has been distributed on CD Rom to the member states.

1 Background information

1.1 Identity of the active substance

1.1.1 Common name, synonyms and manufacturer's development code number

Fluoxastrobin (Provisionally ISO accepted), HEC 5725

1.1.2 Chemical name (IIA 1.4)

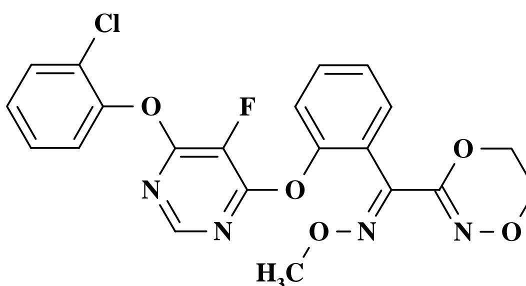
IUPAC: (E) (2-{[6-(2-chlorophenoxy)-5-fluoro-4-pyrimidinyl]oxy}phenyl) (5,6-dihydro-1,4,2-dioxazin-3-yl)methanone O-methyloxime (ACD)

CA: (E) Methanone, [2-[[6-(2-chlorophenoxy)-5-fluoro-4-pyrimidinyl]oxy]phenyl](5,6-dihydro-1,4,2-dioxazin-3-yl)-, O-methyloxime (9CI)

1.1.3 Molecular and structural formulae, molecular mass (IIA 1.7)

Molecular formula: $C_{21}H_{16}ClFN_4O_5$

Molecular structure:



Molecular mass: 458.8 g/mol

1.1.5 Specification of purity of the active substance (IIA 1.9)

Minimum purity - ≥ 940.0 g/kg (HEC 5725 E-isomer)

1.2 Identity of the plant protection product

Trade name: HEC 5725 EC 100 (‘BAYER UK831’)

Emulsifiable concentrate (EC) containing 100g fluoxastrobin/l

1.3 Uses of the plant protection product

Agricultural fungicide.

1.3.3 Summary of intended uses (IIA 3.4; IIIA 3.3, 3.4, 3.5, 3.6, 3.7, 3.9)

Details of intended uses (GAP information) for HEC 5725 EC 100

Crop and/ or situation (a)	**Member State or Country**	**Product name**	**F G or I** (b)	**Pests or Group of pests controlled** (c)	**Formulation**		**Application**				**Application rate per treatment**			**PHI** (days) (l)	**Remarks** (m)
					Type (d-f)	Conc. of a.s. (i)	method kind (f-h)	growth stage & season (j)	number min max (k)	interval between applications (min)	% product min max (n)	water L/ha min max	kg a.s./ha min max		
Wheat, rye, barley	EU North South	not defined	F	Rusts, Leave spot. Pyren. teres, Powd. mildew, Rhynchospor., Septoria	EC	100 g/L	overall spray	start 26 up to BBCH 69	1 – 2 #	ref. to growth stage		200 - 400	0.1 - 0.2	35	# number application depends on disease incidence

Remarks:

(a) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (*e.g.* fumigation of a structure)
(b) Outdoor or field use (F), glasshouse application (G) or indoor application (I)
(c) *e.g.* biting and sucking insects, soil born insects, foliar fungi, weeds
(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)
(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989
(f) All abbreviations used must be explained
(g) Method, *e.g.* high volume spraying, Low volume spraying, spreading, dusting, drench
(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated
(i) g/kg or g/L
(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application
(k) The minimum and maximum number of application possible under practical conditions of use must be provided
(L) PHI - minimum pre-harvest interval
(m) Remarks may include: Extent of use/economic importance/restrictions
(n) product concentration of spray liquid

2. Physical and chemical properties

Shelf life and storage stability of the formulation (IIIA 2.7)

HEC 5725 EC 100 ('BAYER UK831'), an emulsifiable concentrate (EC) containing 100g fluoxastrobin /l was stored for two years at ambient temperature in HDPE and COEX / EVAL commercial packaging. There were no significant changes in active substance content, appearance, pH and emulsion stability. The data submitted indicated that the formulation was chemically and physically stable for 2 years at ambient temperatures.

Guldner, 2003a and b

Method of analysis for the active substance in the plant protection product (IIIA 5.1)

A weighed portion of the EC100 formulation was diluted with acetonitrile and the resulting solution analysed by reverse phase HPLC –UV (254 nm), using a RP 8 column. Validation data are shown in the table below. Representative chromatograms were submitted and were acceptable.

	linearity (linear between) (g/l)	precision – repeatability (%RSD)	accuracy (%)	interference
Fluoxastrobin	0 – 0.06	0.3 (n = 14)	100	No interference

(Seidel 2000 and Odendahi 2000)

3 Toxicology and metabolism

3.1 Toxicological assessment of the new technical specification for fluoxastrobin

The new technical specification for which approval is sought has a minimum purity of 94% and contains 10 impurities (excluding water and sulphated ash), of which 6 were not included in the former technical specification.

The applicant has provided the following additional studies to support the new technical specification, and to satisfy certain of the data requirements outlined in the original DAR:

- a skin sensitisation study with a batch of fluoxastrobin (batch 898109908, 93.6% pure) containing many of the impurities in technical specification for which approval is sought. The batch (Batch NLL 6112-4, 98.1% pure) used for the skin sensitisation study originally submitted was of high purity and no evidence is provided of it containing any of the impurities in the new proposed technical specification. The composition of both of these batches is given at Table C.1.2.2.in Addendum2. (Batch NLL 6112-4 has been re-analysed, see note 2 below).

Of the 10 impurities (excluding water and sulphated ash) in the new proposed technical specification, seven are present in the batch used for the new skin sensitisation study (5 are at a lower concentration in the tested batch than in the proposed technical specification).

- toxicity studies (acute oral and Ames) for 6 of the impurities in the new proposed technical specification. The identity of these impurities is given in Table C.1.1.in Addendum 2).

Notes:

1) The Z isomer of the active substance is now included as impurity no 1. Therefore, the numbering of impurities has now been revised from that previously presented in Volume 4 of the DAR. Volume 4 of the DAR has been updated in the light of the new data and is presented as DAR addendum 2.

2) The following batches used for toxicity studies in the original DAR have been re-analysed (results of the re-analysis, along with the the original analysis for other batches used for the toxicity studies, are presented at C.1.2.2 in Addendum 2:

   - Batch 06261/0008: used for many studies including all dog studies and long term studies in rodents

   - Batch NLL 6112-4: used for several studies including the originally submitted skin sensitisation study and some genotoxicity studies

   - Batch NLL 6112-8: only used for the rat developmental toxicity study

A full list of the studies conducted on these batches is at Table B.6.1 in the original DAR.

3.1.1 Skin sensitisation study with technical material

Guidelines: The study mostly complies with OECD guideline 406 (1992). However the guideline recommends for non-irritant substances pre-treatment with sodium lauryl sulfate (SLS) before topical induction. Although there was no SLS pre-treatment in this study, this is considered acceptable because ECETOC (2000) have recommended that SLS is no longer used as a pre-treatment in the guinea pig maximisation test.
GLP: Yes
Material and methods: HEC 5725 (batch: 898 109 908, purity: 93.6%) was tested in the Maximization Test of Magnusson and Kligman at the following concentrations:
5% for intradermal induction,
50% for topical induction and challenge.

HEC 5725 was formulated as a suspension in physiological saline solution with the aid of Cremophor EL 2% (v/v). Guinea pigs (Crl:HA strain) were used: 20 in the test group and 10 in the negative control group.
Dose levels were based on a sighting study (skin responses were seen following intradermal injection of 1-5%, none seen following topical application of up to 50%).
Findings: After intradermal induction the animals of the test substance group showed strong effects (up to encrustation at the injection sites of the first induction).
Challenge with 50% HEC 5725 led to no skin effects in the test substance group nor in the control group.
A reliability check reported a few months before this study with guinea pigs of an apparently different strain treated with alpha-hexylzimtaldehyd gave a clear positive result.

Conclusions: HEC 5725 (93.6% pure) has no skin-sensitisation potential in the maximization test. This result is consistent with the lack of skin sensitisation potential observed with the HEC 5725 of high purity (98.1%).

(Vohr, 2003)

3.1.2 Acute oral toxicity studies with impurities

a) Acute oral toxicity test with impurity 7

Guidelines: Study complied with OECD 423 (2002). **GLP:** Yes

Material and methods: Impurity 7 (batch: HEC 6148-3-3, purity: 99.2%) in demineralised water with the aid of Cremophor EL 2% (v/v) was administered as a single dose by gavage to fasted Wistar rats (HsdCpb:WU (SPF-bred)). The dose was 2000 mg/kg bw and the application volume was 10 mL/kg bw. There were 3 animals/sex.
Findings: No mortalities occurred and no clinical signs were observed. The study investigators considered that there were no toxicological effects on body weight or body weight gain. No treatment-related findings were noted at gross necropsy.

Conclusion: Impurity 7 has a very low acute oral toxicity in rats (LD 50 > 2500 mg/kg bw, according to OECD guidelines).

(Krötlinger 2002a)

(b) Acute oral toxicity test with impurity 15

Guidelines: Study complied with OECD 423 (2002).

GLP: After completion of the study the original study plan was discarded by mistake by archive staff. However, the electronic template is still available and the existence of an approved study plan can be shown through Quality Assurance records. Hence this deviation from GLP is considered to not invalidate the study.

Material and methods: Impurity 15 (batch M21860, purity: 98.9%) was administered as a single dose by gavage to fasted Wistar rats (3 /sex) at 2000 mg/kg bw. The methodology (including vehicle) was the same as for study a) above.

Findings: No mortalities occurred and no clinical signs were observed. The study investigators considered that there were no toxicological effects on body weight or body weight gain. No treatment-related findings were noted at gross necropsy. Homogeneity of the formulated material was marginally outside defined limits but this does not compromise the value of the study.

Conclusion: Impurity 15 has a very low acute oral toxicity in rats (LD 50 > 2500 mg/kg bw, according to OECD guidelines).

(Krötlinger 2003a)

(c) Acute oral toxicity test with impurity 20

Guidelines: Study complied with OECD 423 (2002). **GLP:** Yes

Material and methods: Impurity 20 (batch KTS 9870-1-2, purity: 99.1%) was administered as a single dose by gavage to fasted Wistar rats (3/sex) at 2000 mg/kg bw. The methodology (including vehicle) was the same as for study a) above.

Findings: No mortalities occurred and no clinical signs were observed. The study investigators considered that there were no toxicological effects on body weight or body weight gain. No treatment-related findings were noted at gross necropsy.

Conclusion: Impurity 20 has a very low acute oral toxicity in rats (LD 50 > 2500 mg/kg bw, according to OECD guidelines).

(Krötlinger 2002b)

(d) Acute oral toxicity test with impurity 21

Guidelines: Study complied with OECD 423 (2002). **GLP:** Yes

Material and methods: Impurity 21 (batch HEC 5641-4-8, purity: 99.0%) was administered as a single dose by gavage to fasted Wistar rats (3/sex) at 2000 mg/kg bw. The methodology (including vehicle) was the same as for study a) above.

Findings: No mortalities occurred. Diarrhea was seen in males 5h after dosing. The study investigators considered that there were no toxicological effects on body weight or body weight gain. No treatment-related findings were noted at gross necropsy.

Conclusion: Impurity 21 has a very low acute oral toxicity in rats (LD 50 > 2500 mg/kg bw, according to OECD guidelines)..

(Krötlinger 2003b)

(e) Acute oral toxicity test with impurity 22

Guidelines: Study mostly complied with OECD 423 (2002) but day-8 body weights were not recorded for males. This is considered to be not a critical omission.
GLP: Yes

Material and methods: Impurity 22 (batch KTS 9872-10-3, purity: 98.6%) was administered as a single dose to fasted Wistar rats (3/sex) at 2000 mg/kg bw. The methodology (including vehicle) was the same as for study a) above.

Findings: No mortalities occurred and no clinical signs were observed. The study investigators considered that there were no toxicological effects on body weight or body weight gain. No treatment-related findings were noted at gross necropsy.

Conclusion: Impurity 22 has a very low acute oral toxicity in rats (LD 50 > 2500 mg/kg bw, according to OECD guidelines).

(Krötlinger 2003c)

(f) Acute oral toxicity test with impurity 23

Guidelines: Study complied with OECD 423 (1996). The use of a dose of 200 mg/kg bw (rather than 300 mg/kg bw) means that the study does not comply with the updated

guideline (2002). However this does not limit the value of the study for assessing the acute toxicity of impurity 23. **GLP:** Yes

Material and methods: Impurity 23 (batch MUEH12/19, purity: 94.5%) in demineralised water with the aid of Cremophor EL 2% (v/v) was administered in a single dose by gavage to fasted Wistar rats (HsdCpb:WU (SPF-bred)). A dose of 200 mg/kg bw was administered to 3/sex and 2000 mg/kg bw to 3 females. The application volume was 10 mL/kg bw.

Findings:

Dose [mg/kg bw]	Toxicological results#	Duration of signs	Time of death	Mortality [%]
males				
200	0 / 1 / 3	1h – 3h	-	0
females				
200	0 / 3 / 3	20min – 3h	-	0
2000	3 / 3 / 3	20min – 5h	2h – 5h	100
LD_{50}: >300 < 500 mg/kg bw##				

\# number of dead animals/ number of animals with signs / number of animals in the group
\## according to the OECD Guideline 423

Clinical signs, body weights: At, 200 mg/kg bw there were no deaths but females showed decreased motility and increased salivation; in one male only motility was decreased. The study investigators considered that there was no substance-related effect on body weight or body weight gain at this dose. At 2000 mg/kg bw, females exhibited increased motility, uncoordinated gait, labored breathing, increased salivation (in one female red discolored), piloerection, lateral position, temporary convulsions and clonic cramps. All females died within five hours after administration.

Gross necropsy: No treatment-related findings were observed at the end of the post treatment ovservation period. In animals which died during the observation period, dark-red discolored livers and slightly collapsed lungs were observed.
Conclusion: Impurity 23 has a moderate acute oral toxicity in rats, with an LD50 of >300 < 500 mg/kg bw according to OECD guidelines.

(Krötlinger 2002c)

3.1.3 Genotoxicity studies with impurities

(a) Ames test with impurity 7

Guidelines: Study complied with OECD guideline 471 (1997). **GLP:** Yes

Material and methods: Impurity 7 (batch HEC 6148-3-3, purity: 99.2%) as a solution in DMSO was initially tested in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. Substance precipitation started at 1600 µg/plate. Therefore, 5000 µg/plate could not be used for assessment (the study investigator indicated that no evaluation was possible at this dose) and highest dose used for assessment was 1581 µg/plate. The Salmonella strains were the histidine-auxotrophic strains TA 1535, TA 100, TA 1537, TA 98 and TA 102. Historical control data were provided for all strains.

All experiments were conducted with and without Aroclor 1254 induced rat liver S9 and included solvent controls (no untreated controls).

The independent repeat assay was performed with pre-incubation for 20 minutes at 37°C with a top dose of 3200 µg/tube; other conditions were as in the plate incorporation assay.

Sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine, mitomycin C, cumene hydroperoxide and 2-aminoanthracene were used as positive controls.

Impurity 7 was shown to be stable in DMSO for 4h at room temperature, ie for a period of time that covers the period from dose preparation to last treatment of plates.

Statistical analysis was limited to calculation of means and standard deviations.

Findings: There was no convincing evidence from bacterial counts (with S9) or revertant counts of a toxic response and no inhibition of growth was noted. Precipitation at 5000 µg/plate would appear to have prevented evaluation of the number of revertants per plate in the plate incorporation assay. Precipitation at 1600 and 3200 µg/plate in the pre-incubation assay did not prevent evaluation of the number of revertants.

The study investigator considered that impurity 7 concentrations up to 3200 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. The RMS notes that there was a 1.7 times increase in the number of revertants with TA 1537 at 1581 µg/plate in the absence of S9 but this increase was mainly due the response on one plate and the overall increase was well short of the laboratory's criterion for a positive response in this strain (at least a 3 times increase) and there was no increase at doses up to 3200 µg/plate in the pre-incubation assay. Hence the RMS agrees that impurity 7 was non-mutagenic in this study.
Positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 7 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

(Herbold 2002a)

(b) Ames test with impurity 15

Guidelines: Study complied with OECD guideline 471 (1997)**. GLP:** Yes .

Material and methods: Impurity 15 (batch M21860, purity: 98.9%) was tested as a solution in DMSO in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. An independent repeat assay was performed with pre-incubation up to and including 5000 µg/plate. All other methodology was as described for study a) above. Impurity 15 was shown to be stable in DMSO for 4h at room temperature.

Findings: Precipitation was seen at 1581 µg/plate and above. There was evidence of toxicity at 5000 µg/plate for strain 102 in the presence of S9 in the plate incorporation

assay (reduction in revertant count and bacterial titer). Impurity 15 concentrations up to 5000 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. The positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 15 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

(Herbold 2002b)

c) Ames test with impurity 20

Guidelines: Study complied with OECD guideline 471 (1997). **GLP:** Yes.

Material and methods: Impurity 20 (batch KTS 9870-1-2, purity: 99.1%) was tested as a solution in DMSO in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. An independent repeat assay was performed with pre-incubation up to and including 5000 µg/plate. All other methodology was as described for study a) above. Impurity 20 was shown to be stable in DMSO for 4h at room temperature

Findings: Precipitation was seen at 1581 µg/plate and above. The only evidence for a toxic response was a slight reduction in the number of revertant colonies for TA102 at 5000 µg/plate in the plate incorporation assay (with and without S9). Impurity 20 concentrations up to 5000 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. The positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 20 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

(Herbold 2002c)

(d) Ames test with impurity 21

Guidelines: Study complied with OECD guideline 471 (1997) **GLP:** Yes.

Material and methods: Impurity 21 (batch HEC 5641-4-8, purity: 99%) was tested as a solution in DMSO in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. An independent repeat assay was performed with pre-incubation up to and including 5000 µg/plate. All other methodology was as described for study a) above. Impurity 21 was shown to be stable in DMSO for 4h at room temperature.

Findings: Precipitation was seen at 1581 µg/plate and above. There was questionable evidence for slight toxicity in a few instances at the top dose (as evidenced by a slight reduction in revertant count). Impurity 21 concentrations up to 5000 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. The positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 21 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the preincubation modification of the Salmonella/microsome test.

(Herbold 2002d)

(e) Ames test with impurity 22

Guidelines: Study complied with OECD guideline 471 (1997). **GLP:** Yes

Material and methods: Impurity 22 (batch KTS 9872-10-3, purity: 98.6%) dissolved in DMSO was tested in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. An independent repeat assay was performed with pre-incubation up to and including 1581 µg/plate. All other methodology was as described for study a) above. Impurity 22 was shown to be stable in DMSO for 4h at room temperature.

Findings: Precipitation was seen at 1581 µg/plate and above. It would seem that in some strains (in the plate incorporation assay) precipitation at 5000 µg/plate did not allow evaluation of revertants; in other strains there was evidence of a toxic response (clear reduction in revertant count) at 5000 µg/plate. The study investigator concluded that impurity 22 at up to 1581 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. The RMS notes that in strain TA 1537, in the presence of S9, mutant frequency increased by 1.4-1.5 times at concentrations of 158 and 500 µg/plate (plate incorporation) and by 1.6 –1.9 times at 50 and 158 µg/plate (pre-incubation). However, there was no increase at higher concentrations (notably at 500 µg/plate in the pre-incubation assay where no precipitation or evidence of toxicity was seen). Also the increases were clearly less the laboratory's criterion (a 3-fold increase) for a positive response in this strain. The RMS also notes that the apparently higher revertant frequencies were comparable to historical control data for DMSO and/or water controls. Hence overall the RMS agrees that there was not a mutagenic response in this strain. The positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 22 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

(Herbold 2003a)

(f) Ames test with impurity 23

Guidelines: Study complied with OECD guideline 471 (1997). **GLP:** Yes.

Material and methods: Impurity 23 (batch MUEH12/24/FRAK5, purity: 99.1%) dissolved in DMSO was tested in this Salmonella/microsome assay, using the plate incorporation technique, at concentrations of up to and including 5000 µg/plate. An independent repeat assay was performed with pre-incubation up to and including 486 µg/plate (toxicity in the plate incorporation assay resulted in lower concentrations being tested in the pre-incubation assay). All other methodology was as described for study a) above, except that mitomycin C was not used as a positive control. Impurity 23 was shown to be stable in DMSO for 4h at room temperature

Findings: No precipitation was reported.

Toxicity

In the plate incorporation assay, toxicity was seen in all strains with and without S9 at 500 µg/plate and above, as evidenced by a reduction in background lawn and/or a reduction in number of revertants and/or reduction in bacterial titer (later only assessed in presence of S9). The study investigator also noted a reduction in bacterial titer at 158 µg/plate for strain TA 100 and TA 98 in presence of S9.

In the pre-incubation assay, toxicity was seen in all strains with and without S9 at 162 µg/plate and above, as evidenced by a reduction in background lawn and/or a reduction in number of revertants and/or reduction in bacterial titer (later only assessed in presence of S9). There was also a reduction in background lawn for TA1537 at 54 µg/plate in the absence of S9.

Genotoxicity

Impurity 23 concentrations up to 500 µg/plate with and without S9 mix did not produce a biologically relevant increase in the mutant count. Assessment at higher concentrations was limited by the toxicity of impurity 23. The positive controls demonstrated a good sensitivity of the assay.

Conclusions: Impurity 23 is considered to be non-mutagenic without and with metabolic activation in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

(Herbold 2002e)

3.1.4 Summary of toxicity studies with impurities of fluoxastrobin

A summary of the toxicity studies with 6 of the impurities in the new proposed technical specification of fluoxastrobin is presented in Table 3.1

All the impurities were of low acute oral toxicity in the rat, except impurity 23 which had an LD 50 of >300 but < 500 mg/kg bw. All impurities were negative in the Ames test, but it is notable (in relation to the rat LD50 result) that impurity 23 was clearly the most toxic to bacteria.

Table 3.1 Summary of toxicity studies with impurities of fluoxastrobin.

Test substance (purity)	Type of study	Organism/Cells	Dose range tested	Result	Reference
Impurity 7 (99.2% pure)	Acute oral	Rat	2000 mg/kg bw	LD_{50} > 2500 mg/kg	Krötlinger (2002a)
	Reverse mutation*	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in 1st experiment 100 – 3200 µg/plate in 2nd experiment	Negative	Herbold (2002a)
Impurity 15	Acute oral	Rat	2000 mg/kg bw	LD_{50} > 2500 mg/kg	Krötlinger (2003a)

Test substance (purity)	Type of study	Organism/Cells	Dose range tested	Result	Reference
(98.9% pure)	Reverse mutation*	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in both experiments	Negative	Herbold (2002b)
Impurity 20 (99.1% pure)	Acute oral	Rat	2000 mg/kg bw	LD_{50} > 2500 mg/kg	Krötlinger (2002b)
	Reverse mutation*	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in both experiments	Negative	Herbold (2002c)
Impurity 21 (99.0% pure)	Acute oral	Rat	2000 mg/kg bw	LD_{50} > 2500 mg/kg	Krötlinger (2003b)
	Reverse mutation*	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in both experiments	Negative	Herbold (2002d)
Impurity 22 (98.6% pure)	Acute oral	Rat	2000 mg/kg bw	LD_{50} > 2500 mg/kg	Krötlinger (2003)
	Reverse mutation	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in 1st experiment 5 – 1581 µg/plate in 2nd experiment	Negative (very slight increase in revertants in TA1537 in 2 assays not considered a mutagenic response)	Herbold (2003a)
Impurity 23 (94.5% pure for acute oral) (99.1% for Ames)	Acute oral	Rat	200 and 2000 mg/kg bw	LD_{50} > 300 <500mg/kg	Krötlinger (2002c)
	Reverse mutation*	*S. typhimurium* TA 1535,1537,98, 100 and 102	16 – 5000 µg/plate in 1st experiment 2 – 486 µg/plate in 2nd experiment	Negative	Herbold (2002e)

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

3.1.5 Toxicological assessment of individual impurities in the new technical specification

The impurity profile of the new proposed technical specification differs from the impurity profile of the former proposed technical specification (see Table C.1.2.2 in Addendum 2). Notably, in comparison with the previous technical specification, the new specification includes some new impurities but also lower maximum levels of other impurities; only one impurity (apart from water) has an increased maximum level. Fourteen impurities in the former specification are not included in the new specification, ie impurities 2, 3, 5, 6, 8, 9, 10, 11, 12,13, 14, 16,17,18. The specified minimum content of a.s in the new specification is also slightly greater than in the previous specification.

A toxicological assessment is provided below of individual impurities in the new proposed technical specification. This assessment takes account of the available

toxicity data to support the maximum levels for these impurities in the new proposed technical specification.

With the exception of impurity 1, all other impurities in the new proposed technical specification were present at <1%. **At concentrations of <1%, the RMS is concerned principally with the potential for genotoxic activity.** It is relevant that impurities in the new technical specification appear to contain no structural alerts for DNA reactivity according to the model of Tennant and Ashby (1991). Also, it is noteworthy that none of the impurities in the proposed new technical specification (nor in the former proposed technical specification) are structures well known to be of particular toxicological concern at these extremely low concentrations.

Impurity 1 (HEC 5725-Z-isomer)
This impurity is specified now at max. 2.0% (max. 20 g/kg), which is lower than in the previously proposed specification (max 3%). This impurity is contained in the following tested batches at:

06261/0008 : 1.13%,

NLL 6112-24: 8%

HUW 4202-3-3: 8%

898109908: 3.52%.

The applicant notes that:

> "With the exception of the cytogenetics *in vitro* and the embryotoxicity rat study all other relevant toxicological studies were conducted with the composite batch 06261/0008 or with batch HUW 4202-3-3 (Ames test), and batch 898109908 (new skin sensitisation study: result negative). Mutagenicity and reproductive toxicity endpoints are adequately addressed by the micronucleus test, the embryotoxicity study in rabbits, and the 2-generation study in rats which were conducted with the composite batch 06261/0008. Therefore, the specified max. 2.0% (max. 20 g/kg) is considered to be covered by the available toxicological studies".

The RMS notes the similarity in structure to the a.s. The RMS agrees that for the 3 areas of particular concern from the presence of an impurity at 2% (skin sensitisation, genotoxicity and reproductive toxicity) there is sufficient data to support the maximum level of this impurity in the new technical specification.

Impurity 4
This impurity is now specified at max. 0.4% (max. 4 g/kg) which is lower than in the previously proposed specification (max 1%).
This impurity is contained in the composite batch 06261/0008 used for toxicological testing at a concentration of 0.48%.

The applicant notes that:

> "With the exception of the Ames test, cytogenetics *in vitro*, and the embryotoxicity rat study all other relevant toxicological studies were conducted with the composite batch 06261/0008. Mutagenicity and reproductive toxicity endpoints are adequately

covered by the additional HPRT and the micronucleus test, the embryotoxicity study in rabbits, and the 2-generation study in rats which were conducted with the composite batch 06261/0008. Therefore, the specified max. 0.4% (max. 4 g/kg) is considered to be covered by the available toxicological studies".

The lack of any Ames test data for this impurity is of some concern (the Ames test is considered to be of high sensitivity for detecting genotoxic impurities), especially as there are some concerns over the sensitivity of HPRT assays. However it is noted that the E-isomer of impurity 4 is a metabolite of fluoxastrobin in the rat (M74) and therefore there might have been some formation of the E-isomer in the two Ames tests with HEC 5725 (which were both negative). Overall, the RMS considers that there are limitations to the genotoxic assessment of impurity 4. However there is a relatively low maximum level in the proposed technical specification (0.4%). Also no oncogenic effect, or adverse effect on reproductive outcome in the multigeneration study, was seen with batch 06261/0008 (containing 0.48% of this impurity). Hence, the maximum level for impurity 4 in the new proposed technical specification is considered to be acceptable.

Impurity 7

This impurity is specified now at max. 0.6% (max. 6 g/kg) which is lower than in the previously proposed specification (max 0.7%). This impurity is contained in the composite batch 06261/0008 used for toxicological testing at a concentration of 0.46%. The applicant notes that:

With the exception of the Ames test, the cytogenetics *in vitro* and the embryotoxicity rat study all other relevant toxicological studies were conducted with the composite batch 06261/0008. Mutagenicity and reproductive toxicity endpoints are adequately covered by the micronucleus test, the embryotoxicity study in rabbits, and the 2-generation study in rats which were conducted with the composite batch 06261/0008. In addition, impurity 7 (purity: 99.2%) was non-mutagenic in an Ames test and of low acute oral toxicity to rats (LD_{50} >2500 mg/kg bw). Hence the specified max. 0.6% (max. 6 g/kg) content of impurity 7 is considered to be toxicologically acceptable

The RMS agrees that the maximum level for impurity 7 in the new proposed technical specification is acceptable.

Impurity 15

This impurity is specified now at max. 0.8% (max. 8 g/kg) which is higher than in the previously proposed specification (max 0.2%). This impurity is contained in composite batch 06261/0008 at 0.07%. The applicant notes that:

The increased level triggered the conduct of an Ames test and an acute oral rat study with impurity 15 (purity: 98.9%). Based on the results from these studies (Ames test: non-mutagenic, acute oral rat: LD_{50} >2500 mg/kg bw, the specified max. 0.8% (max. 8 g/kg) content of impurity 15 is considered to be toxicologically acceptable.

The RMS considers that the negative Ames test with impurity 15 provides adequate reassurance that this impurity is not genotoxic. Hence the maximum level for impurity 15 in the new proposed technical specification is acceptable.

Impurity 20

This is a new impurity specified at max. 0.8% (max. 8 g/kg) and was not contained in the batches used for toxicological testing (except for a 0.24% content in batch 898109908 used for the additional skin sensitization study).
The applicant notes that:

> Impurity 20 (purity: 99.1%) was therefore tested in an Ames test and in an acute oral rat study. Based on the results from these studies (Ames test: non-mutagenic, acute oral rat: LD_{50} >2500 mg/kg bw), the specified max. 0.8% content of impurity 20 is considered to be toxicologically acceptable.

The RMS considers that the negative Ames test with impurity 20 provides adequate reassurance that this impurity is not genotoxic. Hence the maximum level for impurity 20 in the new proposed technical specification is acceptable.

Impurity 21

This is a new impurity specified at max. 0.8% (max. 8 g/kg) and was not contained in the batches used for toxicological testing (except for a 0.16% content in batch 898109908 used for the additional skin sensitization study). The applicant notes that:

> Impurity 21 (purity: 99.0%) was therefore tested in an Ames test and in an acute oral rat study. Based on the results from these studies (Ames test: non-mutagenic, acute oral rat: LD_{50} >2500 mg/kg bw), the specified max. 0.8% content of impurity 21 is considered to be toxicologically acceptable.

The RMS considers that the negative Ames test with impurity 21 provides adequate reassurance that this impurity is not genotoxic. Hence the maximum level for impurity 21 in the new proposed technical specification is acceptable.

Impurity 22

This is a new impurity specified at max. 0.6% (max. 6 g/kg) and was not contained in the batches used for toxicological testing (except for a 0.11% content in batch 898109908 used for the additional skin sensitization study). The applicant notes that:

> This impurity (purity: 98.6%) was therefore tested in an Ames test and in an acute oral rat study. Based on the results from these studies (Ames test: non-mutagenic, acute oral rat: LD_{50} >2500 mg/kg bw), the specified max. 0.6% content of impurity 22 in the technical HEC 5725 is considered to be toxicologically acceptable.

The RMS considers that the negative Ames test with impurity 22 provides adequate reassurance that this impurity is not genotoxic. Hence the maximum level for impurity 22 in the new proposed technical specification is acceptable.

Impurity 23

This is a new impurity specified at max. 0.5% (max. 5 g/kg) and was not contained in the batches used for toxicological testing. The applicant notes that:

> This impurity was therefore tested in an Ames test (purity: 99.1%) and in an acute oral rat study (purity: 94.5%). Based on the results from these studies (Ames test: non-mutagenic, acute oral rat: LD_{50} >300 <500 mg/kg bw), the specified max. 0.5% content of impurity 23 is considered to be toxicologically acceptable.

The RMS considers that the negative Ames test with impurity 23 provides adequate reassurance that this impurity is not genotoxic. Although this impurity is more acutely toxic by the oral route than the active substance, at a concentration of 0.5% it would have no significant impact on the acute toxicity of the technical grade a.s., ie the LD50 of the technical grade a.s. would be > 2,500 mg/kg bw. It is also reassuring for consumer risk assessment that this impurity is not reported to be a metabolite of the a.s. in plant or animals (see Appendix 3.4 in original DAR). Hence the maximum level for impurity 23 in the new proposed technical specification is acceptable.

Impurity 24

This is used as a solvent in the current production process. It was not contained in the batches used for toxicological testing and is now specified at max. 0.5% (max. 5 g/kg). The applicant notes that:

> For this impurity, with the exception of long term data, a complete toxicological data set including human data is available (IUCLID Data Set, created by the European Chemical Bureau (ECB), dated 1995-10-23). According to this data set impurity 24 of low acute toxicity after oral, dermal and inhalation exposure. It is irritant to skin and eye but not skin sensitising. The main target after repeated application on rat and monkey and, to a lower extent, on mouse and dog is the kidney. The impurity showed neither a embryotoxic/teratogenic nor a genotoxic potential. Based on these data the specified max. 0.5% (max. 5 g/kg) content of impurity 24 is considered to be toxicologically acceptable.

The RMS considers that the reported lack of genotoxic potential for this impurity indicates that the maximum level for impurity 24 in the new proposed technical specification is acceptable.

(The RMS notes that this substance is not listed in the UK Approved Supply List, which implements Annex 1 of Directive 67/548).

Impurity 25

This is used as solvent in the new production process. It was not contained in the batches used for toxicological testing and is now specified at max. 0.4% (max 4 g/kg). The applicant notes that:

> For this impurity a complete toxicity data set including human data is available (Deutsche Forschungsgemeinschaft (DFG) 1999, IUCLID Data Set, created by the European Chemical Bureau (ECB), dated 1995-10-23) According to this data set impurity 25 is of low acute toxicity for animals after oral, dermal and inhalation exposure, but harmful for man after acute oral uptake. It has an irritating potential on skin and eye, but is not skin sensitising. The main target after repeated application on rat is the liver. Impurity 25 showed neither a genotoxic nor a carcinogenic potential. In high concentrations developmental toxicity could be observed. However as long as the uptake stays below to the TLV (MAK – value

(270 mg/m³ air)) no harm to the unborn child has to be expected. Based on these data the specified max. 0.4 % (max. 4 g/kg) content of impurity 25 is considered to be toxicologically acceptable.

The RMS considers that the reported lack of genotoxic potential for this impurity indicates that the maximum level for impurity 25 in the new proposed technical specification is acceptable. (The RMS notes that in the UK Approved Supply List, which implements Annex 1 of Directive 67/548, no classification for this substance is required at concentrations of less than 3%.)

Impurity 26 (sulphated ash)

Sulphated ash (sum of inorganic impurities calculated as sulphate ash was contained in the batches of the former MA study below 0.1% (< 1 g/kg) and therefore it was not specified. Based on the current MA-study it is specified at max. 0.5% (max. 5 g/kg). However sulphated ash is an inorganic inert residue and therefore considered to be toxicologically non-relevant.

3.1.6 Commentary on the way the applicant has addressed data requirements relating to impurities

Two data requirements relating to impurities were proposed in the original DAR. Both requirements refer to the need for testing when a final full production batch is available. The applicant has not confirmed that the data provided relate to a final full production batch. However the applicant has changed the method of manufacture and has proposed a new technical specification to reflect the current production process. The data requirements have been addressed in terms of the new proposed technical specification. This is considered to be acceptable.

a) When a final full production batch of fluoxastrobin is available, a further Ames study (with a representative final full production batch) should be conducted to provide additional reassurance that low levels of impurities in fluoxastrobin are not of genotoxic concern.

The applicant has not conducted a further Ames study with a representative full production batch. Instead, separate Ames tests have been conducted with all individual impurities (7, 15, 20, 21, 22, and 23) for which the applicant considered the concentration in the composite batch to be not sufficient. All these Ames tests were negative. Hence, for these impurities a more rigorous investigation of mutagenic potential has been conducted than required by the RMS (the data requirement for these impurities is therefore satisfied).

For impurity 1, there is a negative Ames assay with a batch containing a higher level of the impurity than in the new proposed technical specification. Hence adequate Ames data are available for this impurity.

For impurity 4, although no Ames data are available, there are sufficient other data (including a bone marrow micronucleus assay) to indicate that the low maximum level of this impurity (0.4%) in the new proposed technical specification is not of genotoxic concern.

For the two solvents (impurities 24 and 25), information from an official EC data base is cited by the applicant which indicates that neither solvent is genotoxic.

To conclude: the RMS considers that sufficient data have been provided to address the genotoxic potential of impurities. These data, together with the genotoxicity studies conducted with fluoxastrobin (see Table B.6.25 in the original DAR), provide sufficient evidence to conclude that fluoxastrobin (new proposed technical specification) is not genotoxic.

b) **When a final full production batch of fluoxastrobin is available, the applicant needs to evaluate the toxicological significance of impurities in fluoxastrobin for skin sensitisation.**

The applicant considers that it is necessary only to assess the skin sensitisation potential of impurities specified at concentrations greater than 1% in a technical specification. The RMS essentially agrees with this approach but considers it applies to impurities present at 1% and above; this is consistent with the requirement to classify a substance as a sensitiser if it contains 1% or more of a sensitising impurity (see UK Approved Classification and Labeling Guide which implements Annex VI of 67/548/EEC).

For the new proposed technical specification for fluoxastrobin, only impurity no. 1 is specified at 1% or above (it is specified at a max of 2%). The applicant therefore conducted a skin sensitisation assay (M and K maximisation method) with a batch of fluoxastrobin containing 3.5 % of impurity no. 1 and a negative result was obtained (see 3.1.1). This result is consistent with the lack of skin sensitisation potential reported in the original DAR for HEC 5725 of high purity (98.1% pure).

To conclude: the RMS considers that the data requirement has been satisfactorily addressed and that fluoxastrobin (new proposed technical specification) is not a skin sensitiser.

[It is noteworthy that some reassurance is also available regarding the skin sensitisation potential of impurities present at <1% in the new proposed technical specification:

Good reassurance
- *Impurity 7: present at a higher concentration in the batch tested in the new skin sensitisation study than in the proposed technical specification*
- *Impurities 24 and 25: apparently reported in an official EC data base to be not skin sensitisers*

Limited reassurance
- *Impurities 4, 15, 20, 21 and 22: present at a lower concentration in the batch tested in the new skin sensitisation study than in the proposed technical specification*

No reassurance
- *Impurity 23: below limit of quantification in the batch tested in the new skin sensitisation study.]*

3.1.7 Conclusion regarding the acceptability of the new technical specification for fluoxastrobin

The production process of fluoxastrobin technical has been optimised to result in a higher a.s. content, fewer impurities and with only one impurity (the Z isomer of the a.s.) specified at more than 1%. The new proposed technical specification has 10 impurities (1, 4, 7, 15, 20, 21, 22, 23, and the two organic solvents impurities 24 and 25), plus water and sulphated ash in quantities $\geq$ 0.1% ($\geq$1 g/kg). The toxicological assessment of the new proposed technical specification is based on comparison with the composition of the batches that were originally tested in toxicological studies as well as on the results of additional toxicological studies conducted mainly with individual new impurities.

It is concluded that the new proposed technical specification for fluoxastrobin is acceptable in the context of human risk assessment. No further data are required to address the potential toxicity of impurities.

3.2 Toxicity of soil metabolite M48

Ames test with **M48** (E-isomer)

This study was conducted to GLP and in accordance with OECD guideline 471 (1997)**.** Historical control data were provided for all strains.

M48 (batch M13196, purity: 96.8%) dissolved in DMSO was initially investigated using the Salmonella/microsome plate incorporation test for point mutagenic effects on five Salmonella typhimurium strains (TA1535, TA100, TA1537, TA98 and TA102). Doses of up to 5,000 µg/plate were tested.

The independent repeat assay was performed as pre-incubation for 20 minutes at 37°C; other conditions were as in the plate incorporation assay.

All experiments were conducted with and without Aroclor 1254 induced rat liver S9 and included solvent controls (no untreated controls).

M48 was shown to be stable in DMSO for 4h at room temperature, ie for a period of time that covers the period from dose preparation to the end of treatment of plates.

Statistical analysis was limited to calculation of means and standard deviations.

Findings:

In the plate incorporation assay, the mean number of revertants/plate (200) for the negative control with TA 100 in the presence of S9 was higher than the range of historical control data (median + semi-Q range) reported for the test laboratory (max =170). However this apparently high control value is not considered to be of concern because it was at the top of the range quoted by Maron and Ames (1983) for this strain

without S9, which is compatible with the study investigator's acceptability criteria for the assay.

A slight bacteriotoxic effect (reduction in bacterial titer) was seen in most strains at 5,000 µg/plate in the plate incorporation assay. No substance precipitation was noted.

There was no biologically significant increase in the number of revertants in experiments with M48.

Conclusion: M48 (E-isomer) was non-mutagenic without and with S9 mix in the plate incorporation as well as in the pre-incubation modification of the Salmonella/microsome test.

Herbold (2003b)

Assessment of genotoxicity of **M48**

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.

Overall, the RMS considers that there is sufficient evidence to conclude that M48 is not genotoxic and that further genotoxicity testing of M48 is not necessary*. A full consideration of the potential relevance of M48 in groundwater is presented in Section 5 below.

[***Note**: The EC guidance document for assessment of the toxicological relevance of metabolites in groundwater (Sanco/221/2000 – rev. 10) indicates that metabolites which are predicted to be present in groundwater in excess of 0.1 micrograms/l should be tested in at least **three** *in vitro* genotoxicity assays (ie Ames test, gene mutation in mammalian cells and chromosome aberration test). The RMS does not consider it to be necessary for M48 to be tested in these three assays because of the additional reassurance provided by the negative genotoxicity studies for the parent molecule and the likely *in situ* production of M48 from parent in these studies. It is also relevant that M48 lacks structural alerts for genotoxicity and that the parent molecule was not oncogenic when tested in rats and mice.]

3.3 Toxicological significance of reduced levels of serum ALT and/or AST

3.3.1 Approach taken in original evaluation of fluoxastrobin

The following text appeared in the original evaluation of fluoxastrobin (at the start of section B.6.3 'Short-term toxicity' in the Draft Assessment Report):

"One of the consistent findings in short-term and long-term studies in this evaluation is an effect on serum levels of alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST). Reductions in activity were seen in rats and mice and an increase in activity in dogs. Toxicologists regard an increase in serum activity of these enzymes as indicative of hepatic toxicity but the significance of a decrease in activity is not always so clear.

A reduction in the activity of ALT is a common finding in rats exposed to strobulins (which in some cases has been related to reduced food absorption) and it is not always considered to be an adverse finding. However, in the following rodent studies, a reduction in ALT and/or AST was sometimes accompanied by an indicator of reduced hepatic activity (eg a reduction in serum triglycerides in the 3-month rat study).

In general for this evaluation of the toxicity of fluoxastrobin, a statistically significant reduction in ALT or AST of 30-40% or more, particularly if part of a dose response, is considered to be an indicator of an adverse effect even if not accompanied by histological evidence of hepatotoxicity."

3.3.2 Applicant's position

When invited to comment on the toxicological significance of the reduction in these two liver enzymes, the applicant provided the following response.

"BCS believes still that the reduction of serum aspartate aminotransferase and alanine aminotransferase activities is considered not to represent any toxicologically relevant change per se. These changes are most probably due to a slight alteration in food resorption in the treated animals. Because the assumed slight alteration of alimentary status of the experimental animals did not significantly affect food consumption, and only slight effects on body weight gain were observed (mainly at the highest dose), there is no indication that the decrease in enzyme activities represents an adverse effect.

The RMS mentioned that a reduction of serum triglyceride in the 3 month rat study in combination with the reduction of the enzymes should be regarded as an adverse effect. If one looks more detailed to this effect the following facts can be stated:

- Reduction of serum triglyceride could only be observed in the 3 month feeding study in rats. Neither in the subchronic dog study (Jones, R. D., Elcock, L. E., 2001) nor in the chronic studies of rat (Schladt, L., Ruehl-Fehlert, C., 2001), mouse (Eiben, R., 2001) and dog (Jones, R. D., Hastings, T. F., 2001) this effect was shown, i. e. it is not confirmed either by the other species nor by long term application, where 3 month application was part of the study.
- In addition the control values predominantly in males after 4 weeks (1.75 mmol/l) were high in comparison to historical control values (1.50 mmol/l). At the end of the study more or less an adaptation could be observed (see tables 3.2 and 3.3).
- Considering biological variation all values lie inside the 2 S-range of the historical control values. Furthermore a large variability can be observed within the

historical values indicating that the deviations observed has to be seen more in a statistical than a biological sense.

Summarizing, the pretended reduction of triglyceride in the subchronic oral rat study does not support the reduction of AST and ALT as an adverse effect. In consequence the arguments put forward that the reduction of both enzymes has to be seen as no adverse effects are still valid.

Table 3.2: Clinical chemistry findings in 3-month rat study with HEC 5725 (100% E) Main groups of rats

Dose Ppm	GLUCOSE mmol/1	CHOL mmol/1	TRIGL mmol/1	CREA mcmol/1	UREA mmol/1	BILI-t mcmol/1	PROT g/l	ALBUMIN g/l
Males	Week 4							
0	3.44	2.30	1.75	50	8.89	1.2	64.0	31.4
125	3.41	2.27	1.42	44	8.36	1.3	63.0	31.7
1000	3.44	2.24	1.08++	42+	8.90	1.2	63.3	33.0
8000	3.66	2.03	0.78++	41+	9.56	1.1	63.9	32.6
Males	Week 12/13							
0	3.88	2.38	1.96	50	8.03	1.7	67.4	31.7
125	3.88	2.32	1.68	50	8.41	1.5	67.6	31.7
1000	3.95	2.28	1.78	47	8.48	1.8	68.3	32.3
8000	3.91	2.10	1.23+	48	9.04	1.3++	66.6	33.6++
Females	Week 4							
0	4.07	2.24	1.13	44	8.84	1.3	66.8	33.7
250	4.21	2.39	1.15	43	8.27	1.2	65.0	33.6
2000	4.05	2.33	1.17	43	8.19	1.1	68.4	35.6
16000	4.02	2.27	0.72+	46	8.16	1.2	65.5	35.1
Females	Week 12/13							
0	3.67	2.12	1.65	50	8.82	1.6	67.6	33.7
250	3.75	2.42	1.49	49	8.02	1.7	67.2	33.4
2000	3.87	2.36	1.45	48	8.48	1.4	69.1	35.3
16000	3.79	2.34	1.51	47	8.29	1.4	68.7	35.7

\+ $P \leq 0.05$
++ $P \leq 0.01$

Table 3.3 Historical control data

Bayer AG/Pharma						**Clinical Pathology**			**RAT**	
Breed: Hsd/Cpb WU						Reference values 1998-1999				
Clinical Chemistry						Sampling: Retroorbital veinplexus, not fasted				
Parameter	Age (week) From	to	Sex	N	Mean	Std. Dev	Range -2s	+2s	Range -3s	+3s	Unit
TRIGL	8	11	M	352	1,50	0,491	5P 0,79	2,48	0,03	2,97	mmol/1
TRIGL	8	11	F	369	1,10	0,414	5P 0,54	1,93	up to	2,34	mmol/1
TRIGL	12	25	M	409	1,80	0,619	5P 1,00	3,03	up to	3,65	mmol/1
TRIGL	12	25	F	400	1,51	0.557	5P 0,78	2.63	up to	3,19	mmol/1

3.3.3 RMS comments on the applicant's response

The RMS has not attempted to evaluate fully the company's response because consideration of the toxicological significance of reduced serum ALT and/or AST in rodents does not affect the reference doses (ADI, ARfD and AOEL) set for fluoxastrobin by the RMS in July 2003. These reference doses were all based on effects in dogs (reduced body weight gain and/or increased serum alkaline phosphatase). However, the RMS makes the following observations:

Mechanism of reduced ALT/AST

It is possible that reduced serum ALT and/or AST was related to reduced food resorption, especially as fluxoastrobin has been shown to reduce the phosphate absorption from the intestine. Reduced food resorption has been proposed to explain reductions in ALT and alkaline phosphatase in the serum of rats exposed in the diet to another strobulurin, kresoxim-methyl (see ACP Disclosure Document 163). Notably it is stated in this Disclosure Document that:

> "For rats on normal diets exposure to kresoxim-methyl resulted in a consistent reduction in total and intestinal alkaline phosphatase activity in serum, with a clear reduction in serum alanine aminotransferase activity being seen in some experiments. In starved rats, kresoxim-methyl had little or no effect on serum activities of these two enzymes."

Other investigators have also shown dietary-dependent changes in serum activity of alkaline phosphatase and ALT (see ACP Disclosure Document 163). Additionally, reduced levels of blood ALT and AST have been related to vitamin B6 deficiency and to zinc deficiency (see review by Waner and Nyska 1991). Waner and Nyska point out that until the mechanism of effect of a particular drug or chemical is understood, reduced aminotransferase activities should not be ignored or side stepped as being within normal range or of no biological significance.

Significance of reduced ALT/AST in relation to other possible indicators of reduced hepatic activity

The applicant has commented on one other possible indicator of reduced hepatic activity, reduced serum triglyceride levels (other possible indicators in short-term rat studies were reductions in cytochrome P-450 related enzymes and alkaline phosphatase).

RMS does not agree with the applicant that reduced levels of serum triglycerides were seen only in the 3-month feeding study in rats. This effect was also seen in two of the three 4-week dietary studies with rats. A reduction in serum triglycerides was also seen in high dose males at week 27 (first sample time) in the chronic rat study.

RMS questions whether it is appropriate in this context for the applicant to refer to the absence of an effect on serum triglycerides in dog studies because no reduction in serum ALT or AST was seen in dogs.

It is however true to say that in the chronic mouse study there were marked reductions in ALT (by up to 83%) in the absence of effect on triglycerides.

RMS agrees that the above historical control data for serum triglycerides in rats show notable variability. Also, in the two short-term rat studies reported in detail by the RMS, only at the highest dose levels did reductions in serum triglyceride levels reach the extreme end of the 2-S range for historical control values (after 4 weeks exposure in the 90-day study) or were clearly below this range (at the end of the 4-week study of Andrews). However it is difficult to accept that considerable percentage reductions in serum triglyceride levels (by up to c.50-60%) in these two studies, which were also

seen at a lower dose level (by c.30-40%), are without toxicological significance (even if just as a marker of impaired food / nutrient absorption).

Conclusion

In the original evaluation, the RMS took the view that at some point a reduction in ALT/AST must be of toxicological significance. The RMS proposed that a statistically significant reduction in ALT or AST of 30-40% or more should be considered adverse.

Following further advice from its expert advisory body (the ACP), the RMS now concludes that it was overcautious in interpretation of the toxicological significance of reduced serum ALT and AST, and therefore the observed reductions in ALT/AST were **not** adverse findings in this case. However, the precautionary approach followed in the original DAR has no impact on the reference doses proposed by the RMS. Hence there would not seem a pressing need to reconsider the NOAELs for studies that do not impact on the proposed reference doses. The RMS has however amended the listing of endpoints to reflect the revised view that the reductions in ALT/AST were not adverse findings. The only change necessary to the listing of endpoints was to long term toxicity and carcinogenicity: the target/critical effect has been amended (the critical effect is now reduced body weight) and the NOAEL raised slightly to 35 mg/kg bw/day (previously set at 30 mg/kg bw/day).

The RMS is hoping to develop some guidance (with the help of expert UK advisors) to aid interpretation of reduced serum levels of ALT and AST in future assessments. Therefore, if any members states have already developed such guidance, the RMS would be pleased to receive a copy.

References

Waner T and Nyska A 1991 The toxicological significance of decreased activities of blood alanine and aspartate aminotransferase Veterinary Research Communications 15, 73-78.

4 Residues resulting from supervised trials (IIA 6.3; III 6.5)
Stability of residues prior to analysis

Samples from supervised residue trials were stored frozen for up to 20 months prior to analysis. To confirm that residue samples were stable under the above conditions, samples of tomato, potato, wheat and lettuce were fortified at 0.5 mg/kg with fluoxastrobin (0.45 mg/kg E-isomer and 0.05 mg/kg Z-isomer) and were stored in a freezer (at -18°C) for 30 months. Samples were taken from storage at intervals and analysed by the method described in Section B.5.2a of the Draft Assessment Report. Recoveries are detailed in Table 4.1 and show that residues of fluoxastrobin are stable for up to 31 months in tomatoes, lettuce, wheat forage, wheat grain and wheat straw (Z-isomer which makes up only a small amount of the residue fell slightly below 70% in the 6 and 12 month sample). For both stored and fresh samples of potatoes, recoveries of fluoxastrobin are either just above or just below 70%, indicating that the residues of fluoxastrobin are stable for up to 31 months in potatoes and that the low recoveries were an analytical problem.

(Heinemann 2001, Heinemann 2002 and Heinemann 2003)

Table 4.1 Stability of residues in tomato, potatoes, wheat and lettuce following storage at -18°C

Freezer storage period (months)	Recoveries (%)					
	Tomatoes	Potato	Wheat forage	Wheat grain	Wheat straw	Lettuce
0						
Fluoxastrobin	85	64	80	88	83	89
E-isomer	86	65	80	88	83	89
Z-isomer	81	60	77	92	82	84
2						
Fluoxastrobin	86	72	83	88	77	91
E-isomer	87	72	84	89	78	91
Z-isomer	83	66	82	88	74	89
4						
Fluoxastrobin	87	72	85	92	84	91
E-isomer	87	73	86	92	84	91
Z-isomer	83	66	83	92	80	88
6						
Fluoxastrobin	86	68	85	90	73	85
E-isomer	86	69	85	91	74	86
Z-isomer	81	64	80	86	66	81
12						
Fluoxastrobin	90	70	88	95	74	100
E-isomer	91	71	90	95	75	101
Z-isomer	82	62	77	90	65	89
24						
Fluoxastrobin	85	81	88	91	81	90
E-isomer	86	82	88	91	82	90
Z-isomer	83	77	84	90	73	88
31						
Fluoxastrobin	88	83	82	89	83	88
E-isomer	88	82	81	89	83	88
Z-isomer	93	86	87	93	81	93

Conclusion

Residues of fluoxastrobin are stable for up to 31 months in tomatoes, lettuce, wheat forage, wheat grain, wheat straw and potatoes.

5 Environmental fate and behaviour: Consideration of the potential relevance of metabolite M48 in groundwater

FOCUS-PELMO modelling predicts that levels of the metabolite **M48** (HEC 5725-deschlorophenyl) in percolate at 1m soil depth will be within the range of 0.002-3.65 micrograms/l. (See B.8.5.3.1 of the DAR for fluoxastrobin). FOCUS-MACRO modelling required for UK authorisation also showed that **M48** is expected to exceed 0.1µg/l.

In a series of laboratory test and glasshouse screens, M48 showed virtually no fungicidal properties. In a test on isolated mitochondria of Rice blast (*Pyricularia oryzae*), M48 caused respectively 0 and 6% inhibition at 10^{-4} and 10^{-5}M concentrations compared to 100% from fluoxastrobin. In a radial growth test the EC_{50} for the same pathogen was 70 mg/l for M48 compared to 3.26 mg/l (21.5 times higher) for fluoxastrobin. For all other pathogens tested the toxicity of M48 was too low to determine the EC_{50}, giving minimum response ratios compared to fluoxastrobin of between 18 and 2500.

M48 has no structural alerts for DNA reactivity according to the model of Tennant and Ashby. M48 gave a negative result with the Ames test (See Section 3. above) and all genotoxicity assays with parent gave negative results. M48 is an initial metabolite of fluoxastrobin in the rat and was found at up to 15% of the applied dose in the rat metabolism study. No human health classification is proposed for fluoxastrobin and it is reasonable to expect that no human health classification would be necessary for M48.

No adverse effects were seen in acute toxicity limit tests with fish, aquatic invertebrate and alga species. Data from studies with soil dwelling organisms also indicate that M48 is much less toxic to these species than parent.

On the basis of the available information and in accordance with the guidance document on the relevance of metabolites in groundwater, M48 is considered a **'non-relevant'** metabolite in groundwater. It is not appropriate to include M48 in the definition of residues in groundwater.

For human health risk assessment of M48, the maximum allowable concentration (MAC) in drinking water set for fluoxastrobin (45 micrograms/l) could be considered appropriate for M48. This is supported by M48 being an initial rat metabolite of fluoxastrobin which retains the methoxyimino group responsible for the fungicidal activity of fluoxastrobin via inhibition of mitochondrial respiration (and presumably also for at least some of its toxicological effects). It is also noted that compared with parent the smaller size of M48 and its more polar structure (due to the presence of an OH group) will favour excretion and hence might reduce its potential to cause toxicity.

The predicted level of fluoxastrobin in ground water is <0.001 micrograms/l. Therefore, the sum of parent and M48 predicted in groundwater, 3.65 micrograms/l, is well below the MAC of 45 micrograms/l. Even if a more precautionary MAC for M48 is proposed, based on correcting the MAC for fluoxastrobin for the maximum proportion (15%) of the applied dose of fluoxastrobin reported as M48 in rat excreta

(to some extent this provides a correction for the unknown toxicity of M48), the resulting MAC of 6.75 micrograms/l is greater than the predicted level of M48 in groundwater.

It is appropriate to also consider exposure to M48 via dietary exposure. In metabolism studies, M48 (which was not included in the residue definition for dietary risk assessment) has been identified at low levels in:

- wheat; < 0.01 mg/kg in grain, highest level of 1.1 mg/kg in straw,
- following crops; many values < 0.01 mg/kg, highest level of 0.29 mg/kg in wheat straw planted in soil aged 30 days (but a.s. applied directly to soil and at c. 2N)
- animal products; highest level of 0.19 mg/kg in goat liver after application of at least 30N.

Total NEDIs (based on levels of parent or parent + M55) are all significantly less (<10%) than the ADI. An even greater margin would exist for M48 because in metabolism studies parent was the major component of the residue at harvest in wheat (grain and straw) and in following crops in the 30 day studies, and parent + M55 was the major residue in goat tissues (no major component identified in milk). Notably M48 constituted < 3% of parent in wheat. Not too much reliance should be placed on relative levels of parent and M48 in following crops and goats because there are limitations in the applicability of these metabolism studies for risk assessment.

To conclude, M48 is regarded as a **‘non-relevant’** metabolite in groundwater. The above risk assessment suggests that as the predicted level of M48 in groundwater is no more than 50% of the MAC for parent and the level of M48 in diet is less than 10% of the ADI for parent, there would appear to be no concern for human health from the presence of M48 in groundwater.

6 Ecotoxicology: Effects on aquatic organisms of active substance and formulated product 'HEC 5725 EC 100' (IIA 8.2, IIIA 10.2)

6.1 Background

Fluoxastrobin formulated as an emulsifiable concentrate 'HEC 5725 EC100' (Bayer UK831 containing 100g a.s./l), is proposed for use in cereal crops as a post-emergence spray at the first signs of disease in the autumn or spring / early summer up to before grain milky ripe stage (GS 71). The maximum individual dose would be 200 g a.s./ha and the maximum total dose would be 400 g a.s./ha.

An assessment of the risks to aquatic organisms was presented in the Draft Assessment Report (DAR) for fluoxastrobin (Section B.9.2.5 of Vol. 3). In addition to the standard freshwater species, studies had also been performed on *Americamysis bahia* (marine mysid shrimp), which indicated that this was the most sensitive tested species with respect to effects from long-term exposure. The Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001, October 2002, Section 2.3.3) states that 'In some cases, data are available on estuarine/marine invertebrates (*e.g.*, *Mysidopsis bahia*, oyster embryo larval studies). At present, there is no requirement under Directive 91/414/EEC to perform these studies, but if data are available, they must be submitted and should be considered in the risk assessment.' The Volume 3 DAR therefore includes consideration of these data.

A non-GLP aquatic invertebrate acute toxicity study (Wijngaarden 2003) indicated that certain freshwater invertebrates had a similar sensitivity to fluoxastrobin as the mysid shrimp. When these additional data were taken into account, a 15 metre buffer zone was required to mitigate the chronic risk to aquatic life.

Two further GLP compliant laboratory freshwater aquatic invertebrate toxicity studies have now been conducted so that the aquatic risk assessment can be refined. One of these studies investigates the acute toxicity of fluoxastrobin to a range of freshwater aquatic invertebrates and the other the long-term toxicity of fluoxastrobin to the fresh water aquatic invertebrate *Gammarus pulex*. Details for the two new aquatic toxicity studies are summarised below, together with a revised aquatic risk assessment.

6.2 Acute aquatic toxicity of fluoxastrobin to freshwater aquatic invertebrates

Details for the fluoxastrobin freshwater aquatic invertebrate acute toxicity study are summarised in Table 6.1. These acute toxicity data are additional to those previously summarised in Table B.9.12 of Vol. 3 of the Draft Assessment Report on fluoxastrobin.

The newly submitted data indicate the amphipod *Gammarus pulex* to be the most sensitive tested species, with an acute EC50 of 0.15 mg fluoxastrobin / litre.

Table 6.1: The acute toxicity of technical fluoxastrobin (96-98% purity) to freshwater aquatic invertebrates.

Species	**Test type & duration (nom. dose range mg. tech. a.s./l)[1].**	**Actual conc. (as % of nominal a.s.)**	**Immobilisation EC_{50}* in mg a.s./l (95% CL where calculated)**	**NOEC* mg a.s./l (based on data interpretation)**	**Test Guideline ****	**Ref.**
Invertebrates (IIA 8.2.4)						
Acanthocyclops venustus (adults) #	Static 48 hour immobilisation tests (0.0032-3.2)	At study start: 92-108% At study end: 92-120%	48h: 0.9 (0.4-1.8)	48h: 0.032	**OECD 202 (as far as possible for test species)**	Wijngaarden (2003b)
Gammarus pulex (adults)			48h: 0.15 (0.13-0.18)	48h: 0.032		
Daphnia group *galeata* (adults) #			48h: 1.3 (-)	48h: 1.0		
Cloeon dipterum (nymphs)			48h: 1.0 (0.7-1.6)	48h: 0.1		
Asellus aquaticus (adults)			48h: 1.7 (1.4-2.1)	48h: 1.0		
Chaoborus obscuripes (larvae)##			48h: > 3.2	48h: 0.32		
Simocephalus vetulus (adults)			48h: > 3.2	48h: > 3.2		

* EC50 & NOEC based on nominal concentrations
**Tests conducted broadly in accordance with guideline and to GLP
Test repeated due to significant non-treatment related mortality in initial tests; presented details relate to second test results.
Analysis indicated low level a.s. contamination of *Chaoborus* controls at 48h (i.e. 0.005-0.012 mg a.s./l), but considered unlikely to significantly effect EC50 /NOEC estimates.

6.3 Long-term toxicity of fluoxastrobin to *Gammarus pulex* in a water-sediment 'microcosm' study.

The study aim was to determine the long-term toxicity of fluoxastrobin (applied as 'HEC 5725 100EC) to the freshwater amphipod *Gammarus pulex* when exposed for 28 days in a static water-sediment system.

Methodology

Cylindrical stainless steel tanks with a volume of 18 litres (diameter 26cm and height 35cm) were used as test vessels. Perforated stainless steel inserts (diameter 25cm and height 35cm) were placed inside the tanks onto a 2cm deep layer of sieved natural sediment. The test vessels were each filled with 15 litre of natural ditch water, care being taking to minimise re-suspension of sediment fines.

Forty-five adult *Gammarus pulex* were used per test concentration, fifteen animals being introduced into each of three replicate test vessels. Six nominal (initial) treatment concentrations of 3.2, 10.0, 17.8, 31.6, 56.2 and 100.0 µg a.s./l were included, plus an untreated control. The test item was applied to the water phase of the test system. Gammarids, sediment and water used in the test system were collected outdoors from a source considered to be free of significant pesticide contamination. Poplar leaves (which served as food) were added to the test vessels prior to application of the test item. Treated test vessels at the two highest test concentrations were recolonised with fresh gammarids during the study, with the study period for these vessels being extended to 42 days.

Toxicity endpoints (LC/EC50, NOEC and LOEC) based on mortality and growth rate (biomass) were assessed in comparison to the untreated control.

Water samples from the test systems were taken for analysis 3 hours after application and periodically during the study period (at least once per week), using either LC-MS/MS for the two highest test concentrations ('method 00692') or HPLC-UV for the other lower test concentrations ('method 00579'). The method of analysis was appropriately validated; the percentage relative standard deviations using standard concentrations of 4.86 µg a.s./l and 48.6 µg a.s./l were 0.9-9.4% and 0.3-1.2% respectively.

<u>Results</u>

The analytical determined recovery levels of applied fluoxastrobin in test treatments (as a percentage of nominal values) in the overlying microcosm water were 94.2-109.5% (mean 100.3%) on day 0, 61.3-86.6% (mean 72.9%) on day 14, and 52.5-76.5% (mean 64.2%) on day 28. Recovery levels on day 42 in the analysed two highest test concentrations, showed a further small decrease to 62.6-65.0% (mean 63.8%) of nominal values. It was suggested by the study author that the periodic disturbance of the leaf and sediment layers which occurred when assessing numbers of gammarids, may have led to a slowdown in the decrease of fluoxastrobin concentrations in the water phase of the microcosm. The reported rate of decline in fluoxastrobin levels may not therefore be directly comparable to that in natural systems.

The results of the survival and biomass assessments are summarised in Table 6.2.

Table 6.2 Effects of HEC 5725 EC100 (Bayer UK831), (100g fluoxastrobin /litre) treatment on the survival and biomass of *Gammarus pulex* in the microcosm study.

Nominal conc. µg a.s./l	Mean % survival of gammarids			Mean individual biomass of gammarids	
	Day 4	Day 14	Day 28	Day 0	Day 28
0.0	96	89	89	18.7	27.5
3.2	100	96	93	17.7	25.7
10.0	100	100	100	17.4	26.9
17.8	98	93	91	16.7	30.0
31.6	100	96	82	18.0	29.8
56.2	96	82	71 *	17.4	27.4
100.0	53	4 *	0 *	17.9	-

* Statistical analysis indicates significantly different from untreated control (P<0.05).

Mean mortality in the untreated control was 4% after 4 days, 11% after 14 days, with no further mortality after 28 days.

Treatment at the highest test dose of 100 µg a.s./l resulted in 47% mortality after 4 days, 96% mortality after 14 days and 100% mortality after 28 days. Treatment at 56.2 µg a.s./l resulted in no mortality effects after 4 days, 18% mortality after 14 days and 29% mortality after 28 days. Statistical analysis indicated no significant effect on survival of gammarids at nominal test concentrations of up to 31.6 µg a.s./l, with significant reductions in survival only at 56.2 (after 28 days) and 100 µg a.s./l (after 14 and 28 days).

No treatment related effects on growth rates (based on biomass assessments) were noted during the 28-day exposure period at test doses up to and including 56.2 µg a.s./l. Due to high mortality at 100 µg a.s./l, no assessments of effects on biomass were conducted at this nominal test concentration.

Re-colonisation with fresh gammarids was performed several times after significant mortality occurred at nominal concentrations of 56.2 and 100 µg a.s./l, with the study period being extended to 42 days to assess treatment effects on re-introduced populations.

High levels of mortality of re-introduced populations occurred at the nominal test concentration of 100 µg a.s./l, even when re-introduction occurred as late as 35 days after treatment (where approximately 80% mortality was recorded on day 42).

Following 33% mortality of the initially introduced populations in one replicate at 56.2 µg a.s./l on day 14, the remaining surviving gammarids were removed and replaced with 15 fresh gammarids. A subsequent assessment in this single replicate on study day 42 (28 days after recolonisation) indicated 80% survival (and 20% mortality)

The 28 day 50% lethal effect concentration (LC50) was determined to be 63.6 µg a.s./l and the lowest observed effect concentration (LOEC) as 56.2 µg a.s./l. The no observed effect concentration (NOEC) was 31.6 µg a.s./l, based on a lack of statistically significant effects at this concentration.

Given the reported 80% survival following re-colonisation on day 14 in the one replicate at 56.2 µg a.s./l is 'in the same range of survival' as that occurring over 28 days at 31.6 µg a.s./l (the above determined NOEC), the Notifier has proposed that 56.2 µg a.s./l should be regarded as the 'No Observed Ecological Adverse Effect Concentration' (NOEAEC).

(Liebig M 2003)

6.4 Risk assessment for aquatic life

A revised aquatic risk assessment has been conducted based on consideration of the previously submitted data in combination with the new data. The assessment focuses on the risk to aquatic life from spray drift contamination of surface water from the proposed use of the fluoxastrobin containing formulation 'HEC 5725 EC100' (Bayer UK831). Although fluoxastrobin may also reach surface water from drainflow, the previously conducted assessment (Section B.8.5.1 of Vol.3 of the DAR for fluoxastrobin) indicates that drainflow contamination levels are lower than that from spray drift. Therefore, the risk to aquatic life from drainflow contamination is covered by that for spray drift contamination.

The two new studies are both GLP compliant and conducted to acceptable protocols and as such may be used in the risk assessment.

6.4.1 Acute risk to aquatic life from spray drift

Both the newly submitted GLP compliant aquatic invertebrate acute toxicity study and the previously submitted similar non-GLP study indicate the two most sensitive tested species to be *Acanthocyclops venustus* (48h EC50 new study = 0.9 mg a.s./l, 48h EC50 previous study = 0.03 mg a.s./l) and *Gammarus pulex* (48h EC50 new study = 0.15 mg a.s./l, 48 EC50 previous study = 0.1 mg a.s./l).

It is unclear why the EC50 for *Acanthocyclops venustus* differs so greatly between the two studies (i.e. by a factor of 30) – this not being accounted for by differences in the test doses used in each study. However, given that the previously submitted study was not GLP compliant and did not include any confirmatory analysis of the test concentrations, the EC50 values derived from the more recent GLP compliant study are more reliable and as such should be used in place of the previously submitted data.

Based on the results of the newly submitted acute toxicity study *Gammarus pulex* is the most sensitive tested aquatic invertebrate, with an acute 48h EC50 of 0.15 mg a.s./l. This compares with a previously reported 48h EC50 for the standard test species *Daphnia magna* of 0.48 mg a.s./l and a 96h EC50 for the saltwater mysid shrimp *Americamysis bahia* (<1 day old juveniles) of 0.06 mg a.s./l. *Gammarus pulex* and *Americamysis bahia* are therefore of an approximately similar level of sensitivity to fluoxastrobin (differing by a factor of < 2.3) and both species are significantly more acutely sensitive than *Daphnia magna*.

A comparison of the toxicity endpoints for the most sensitive fish, aquatic invertebrate and plant species with the estimated 1 metre spraydrift exposure levels for the active substance and formulation is presented in Table 6.3. Given the range of freshwater aquatic invertebrate species tested (8 freshwater species and one saltwater species), in line with HARAP guidance (SETAC 1999) and also with the EC Guidance Document on Aquatic Ecotoxicology (SANCO/3268/2001, October 2002), for the most sensitive tested species the Annex VI trigger of 100 may be reduced to 10.

Table 6.3 Acute risk to aquatic life from fluoxastrobin surface water spray drift contamination at 1 metre (overall 90th percentile drift levels)

Species	**LC/EC 50 (mg a.s./l)**	**PEC (mg a.s./l) #**	**TER for a.s.**	**LC/EC 50 for form'n (mg product/l)**	**PEC (mg product /l) ##**	**TER for form'n**	**Annex VI trigger**
Oncorhynchus mykiss (rainbow trout)	0.435	0.00212	205	3.29	0.0185	178	100
Daphnia magna (water flea)	0.48	0.00212	226	5.0	0.0185	270	100
Americamysis bahia (NB. data for 96h EC50 & not standard 48h EC50)	0.06	0.00212	28	-	-	-	10*
Gammarus pulex	0.15	0.00212	71	-	-	-	10*
Pseudo-kirchneriella subcapitata (green alga)	0.3 ♣	0.00212	142	4.8 ♦	0.0185	259	10
Lemna gibba	>6.00	0.00212	2830	-	0.0185	-	10

♣ 96h EbC50 (cell density), ♦ 72 h EbC50.
\# Maximum PEC (a.s.) from spray drift contamination at 1 metre into a 30cm deep static waterbody, as estimated in Section B.8.5.1 of Volume 3.
\## Maximum PEC (formulation) from spray drift contamination at 1 metre into a 30cm deep static waterbody, from one application of 2 litres 'Bayer UK831' /ha, assuming a 90th percentile drift level of 2.77%.
* Annex VI trigger reduced from 100 to a minimum of 10 due to the numbers of tested aquatic invertebrate species permitting a reduction in the uncertainty factor for the most sensitive tested species (as per HARAP 1999 and SANCO 2002 aquatic ecotoxicology guidance).

Conclusion

The acute TERs for the tested fish, aquatic invertebrate, alga and aquatic plant species are all above the relevant (and refined) Annex VI 91/414 trigger values. Therefore the acute risk to aquatic life from spray drift at 1 metre is acceptable.

6.4.2 Long-term risk to aquatic life (including sediment dwellers) from spray drift

Initial risk assessment based on previously submitted data

A comparison of the relevant toxicity endpoints from previously evaluated aquatic long-term toxicity studies with the initial 1 metre spray drift PECsw is included in Table 6.4.

Table 6.4 Long-term risk to aquatic life from fluoxastrobin surface water spray drift contamination at 1 metre (overall 90th percentile drift levels)

Species & study type	**NOEC (mg a.s./l)**	**Initial PEC at 1 m (mg a.s./l) #**	**TER**	**Annex VI trigger**
Fish: *Oncorhynchus mykiss* (95-day early life-cycle study)	0.0286	0.00212	13	10
Aquatic invertebrates: *Daphnia magna* (21-day reproductive toxicity study)	0.18	0.00212	85	10
Americamysis bahia (Mysid shrimp) (28-day life cycle study)	0.00061 (mortality) 0.0047 (reproduction)	0.00212 0.00212	**0.29** **2.2**	10 10
Sediment dwellers: *Chironomus riparius* (28-day sediment-water study)	1.2 ##	0.00317	379	10

\# Maximum PEC (a.s.) from spray drift contamination at 1 metre into a 30cm deep static waterbody, as estimated in Vol.3, Section B.8.5.1.

\## In the absence of a determined NOEC for this study, the lowest EC_5 value has been used (based on effects on development rate).

The toxicity: exposure ratios for the tested standard indicator species of fish, sediment dwellers, and aquatic invertebrates (*Daphnia magna*) are in excess of the Annex VI trigger of 10, indicating an acceptable long-term risk to these organisms. However, the long term mortality and reproductive TERs for the mysid shrimp (*Americamysis bahia)* are in breach of the Annex VI trigger, indicating a potential concern (mortality TER 0.29, reproductive TER 2.2).

It is customary when assessing the long-term risk to aquatic organisms to use, in the first instance, initial predicted environmental concentrations and therefore this approach has been taken in the above assessment. However, an examination of the mysid shrimp chronic toxicity study data (Section B.9.2.4.1 of Vol.3), indicates that the main effects are on mortality and that these effects occurred 16 or more days after initial exposure. It is considered that this effect is the result of prolonged exposure and that it would therefore be more appropriate in the refined risk assessment to compare the mysid shrimp mortality NOEC with the 14 day time weighted average PECsw (as estimated in Section B.8.5.1 of Vol.3 of the DAR for fluoxastrobin). With respect to reproductive effects, it is not possible from the design of the study to determine the time to effect and therefore the reproductive endpoint should be compared (as a worst case) with the initial (maximum) PECsw.

The risk to aquatic life from spray drift contamination may be mitigated by the use of no-spray buffer zones. The effect of use of such buffer zones on the risk to the mysid shrimp (as a sensitive indicator species) is assessed in Table 6.5. As discussed above, the mortality NOEC from the mysid shrimp 28-day chronic toxicity study is compared with the 14-day time weighted average PECsw and the reproductive NOEC with the initial PECsw.

Table 6.5 Effects of use of risk mitigation no spray buffer zones on the long-term risk to aquatic invertebrate species (based on use of chronic toxicity data on the saltwater mysid shrimp as a sensitive indicator species)

Species & study type	**NOEC (mg a.s./l)**	TER at 1m* (initial PEC = 0.00212 mg a.s./l; 14 day twa PEC = 0.0008 **mg a.s./l)**	TER at 5m* (initial PEC = 0.00044 mg a.s./l; 14 day twa PEC = 0.00016 **mg a.s./l)**	**TER at 10m* (initial PEC = 0.00022 mg a.s./l; 14 day twa PEC = 0.00008 mg a.s./l)**	**TER at 15m* (initial PEC = 0.00015 mg a.s./l; 14 day twa PEC = 0.00006 mg a.s./l)**	**TER at 20*m (initial PEC = 0.00011 mg a.s./l; 14 day twa PEC = 0.00004 mg a.s./l)**
Americamysis bahia (Mysid shrimp) (28-day life cycle study)	0.00061 (mortality#)	**0.76**	**3.8**	**7.6**	10.2	15.2
	0.0047 (repro-duction)	**2.2**	10.7	21.4	31.3	42.7

*Based on Northern Europe estimates of surface water spray drift exposure (Section B.8.5.1.1), an initial spray drift PECsw for reproductive effects, and a 14 day t.w.a. PECsw for mortality effects (see text)
Based on mortality effects at higher test doses of $\geq$ 0.00119 mg a.s. /l developing 16 days or more after initial exposure.

Therefore based on the previously submitted mysid shrimp long-term toxicity data, in order for the Annex VI 'acceptability' trigger of 10 not to be breached a minimum of a 15 metre no-spray buffer zone is required.

Further risk assessment taking account of newly submitted data

The initial risk assessment indicates an acceptable long-term risk (without mitigation measures) to the aquatic invertebrate *Daphnia magna* (TER 85) and the sediment dwelling invertebrate *Chironomus riparius* (TER 379). However, the toxicity data for *Americamysis bahia* indicates a potential concern.

In order to address this risk the Notifier has carried out a long-term toxicity study using *Gammarus pulex*. This species was chosen as it had been shown from the results of GLP compliant acute fluoxastrobin toxicity tests to be the most acutely sensitive of the total of eight freshwater aquatic invertebrate species tested. It was also considered relevant and representative of invertebrates in agricultural environments. In addition this amphipod crustacean is related to the sensitive saltwater mysid *Americamysis bahia.*

The *Gammarus* study monitored the effects of fluoxastrobin on mortality and body weight and indicates similar effects to that in the mysid study, with mortality increasing with time. Although only effects on mortality and biomass were assessed with no assessment of reproductive effects, the previously submitted mysid shrimp chronic toxicity study indicates mortality to be a much more sensitive effect parameter than reproduction. Also, a careful analysis of the results of the mysid shrimp chronic toxicity study indicates no increase in adult sensitivity to fluoxastrobin as a result of commencement of reproduction. On this basis, the protocol for the *Gammarus* long-term toxicity study is considered acceptable, with the results obtained being suitable for use in the aquatic long-term risk assessment.

The Notifier has proposed that the nominal initial test concentration of 56.2 μg a.s. /l should be regarded as the 'No Observed Ecological Adverse Effect Concentration' (NOEAEC), based on a lack of significant mortality in a fresh population of 15 gammarids when introduced into one replicate test vessel some 14 days after treatment. However, the Rapporteur considers that data from just one replicate are insufficient to support a NOEAEC. Also, the acceptability of a lack of effect 14 days after treatment at this test concentration assumes the possibility of adequate re-colonisation after this time, which may not be possible in the natural environment. Additionally, differences in the availability of food and in environmental conditions between this laboratory microcosm study and the natural environment, may affect the ability of populations to recover from potential adverse effects of fluoxastrobin. It is therefore considered more appropriate to use the NOEC of 0.0316 mg a.s. /l (based on a lack of statistically significant mortality effects) as the regulatory endpoint from this study.

Using the *Gammarus* NOEC of 0.0316 mg/l and comparing this to the initial 1 metre spray drift PECsw of 0.00212 mg/l produces a TER of 14.9 which is within the Annex VI trigger of 10. Therefore if the risk assessment is based on a relevant, representative and sensitive test species, a safe use can be identified without the need for a buffer zone.

The above risk assessment does not take account of the previously submitted long-term toxicity data for the saltwater mysid shrimp (*Americamysis bahia*). Given that the environmental risk assessment tends to focus on effects at the population level, it could be argued that the mysid shrimp reproductive NOEC of 0.0047 mg a.s. /l is the most relevant for risk assessment purposes. If this endpoint is used, comparison with the 5 metre spraydrift PECsw produces a TER of 10.7 which is within the Annex VI trigger of 10. However, the significance of mortality resulting from prolonged exposure is not known and must be considered. If the 28 day mortality NOEC of 0.00061 mg a.s. /l is compared with the 5 metre spraydrift 14 day twa PECsw of 0.00016 mg/l, the TER is 3.8.

Mean concentrations of fluoxastrobin in the static long-term gammarus study on day 28 (study end) did not fall below 64% of initial values and therefore would not have differed greatly from that present had the study been a flow-through one, as in the long-term salt water mysid shrimp study. Therefore, although differences in methodology does not permit precise comparisons to be made between the two study endpoints, based on the fact that the long-term NOEC reported for the mysid shrimp (0.00061 mg a.s./l) is much lower than that reported for gammarus (long-term NOEC

0.0316 mg a.s./l), it is likely that the mysid shrimp is significantly more sensitive to long-term affects from fluoxastrobin than gammarus.

Given that the mysid shrimp is likely to be amongst the most sensitive of aquatic organisms to fluoxastrobin, a slight lowering of the Annex VI long-term TER acceptability trigger of 10 is permissible. This is supported by Section 5.3 of the 'Guidance Document on Aquatic Ecotoxicology' (SANCO 2002) which states 'The testing of more species reduces the uncertainty of the risk assessment attributable to inter-species differences in sensitivity … It therefore permits a reduction of the uncertainty factor that is applied to the lower-tier data.' On this basis, the estimated mysid shrimp long-term TER of 3.8 obtained using a 5 metre no spray buffer zone is considered sufficient to indicate an acceptable margin of safety to freshwater aquatic invertebrates. Using the chronic toxicity data for *Gammarus pulex* (the next most sensitive tested aquatic invertebrate species) and this proposed 5 metre buffer zone, the long-term TER is 71.8 (which compares with one of 14.9 without risk mitigation).

Conclusion

The proposed use of 'HEC 5725 EC 100' (Bayer UK831) as a fungicide spray in cereals poses a long-term risk to aquatic life (as indicated by the mysid shrimp long-term TER of 0.76). In order for this risk to be reduced to acceptable levels, risk mitigation measures in the form of a 5 metre 'no spray' buffer zone are required.

Appendix 1

List of studies which were submitted during the evaluation process and were not cited in the draft assessment report.

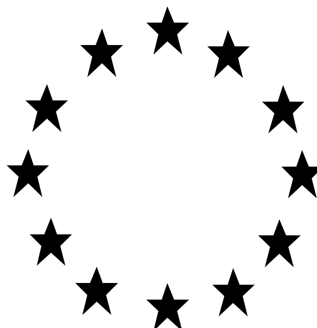
Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not
II A, 1.11 /02	Ruengeler, W.	2004	Material Accountability of Fluoxastrobin (HEC 5725 B) Bayer AG, Report No.: 15-920-2213, MO-04-000576 Date: 21.01.2004 GLP, unpublished C*onfidential*
III A, 2.7.1 /03 EC 100	Gueldner, W.	2003a	Storage stability of HEC 5725 EC 100 - (HDPE) – final report Bayer CropScience AG, Report No.: MO-03-007196, Date:06.06.2003 Non GLP, unpublished
III A, 2.7.1 /04 EC 100	Gueldner, W.	2003b	Storage stability of HEC 5725 EC 100 - (COEX/EVAL) – final report Bayer CropScience AG, Report No.: MO-03-007195, Date:06.06.2003 Non GLP, unpublished
II A, 4.1.2 /05	Hake, G.	2003	Fluoxastrobin Assay of Technical Grade Active Ingredient: HPLC – External standard Bayer CropScience Report No.: AM000103MP1, MO-03-011996 Date: 06.08.2003, Non GLP, unpublished ***Confidenial***
II A, 4.1.2 /06	Hake, G.	2003b	Fluoxastrobin byproducts: HPLC – External standard Bayer CropScience Report No.: AM000303MP1, MO-03-012006 Date: 28.08.2003, Non GLP, unpublished ***Confidenial***
II A, 4.1.3 /09	Krämer, F.	2003	Validaton of GLC-method 2005-0013102-02, Determination of volatile solvents in active ingredient of agrochemicals, GLC – Internal standard Headspace, Specific Solvent: 4-methyl-2-pentanon (MIBK) Bayer CropScience, Report No.: VB1.7-2005-0013102 MO-03-012470 Date: 22.07.2003 Non GLP, unpublished Confidential

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not
II A, 4.1.3 /10	Hake, G.	2003c	Validaton of HPLC-method AM000103MP1, Fluoxastrobin Assay of Technical Grade Active Ingedient Bayer CropScience, Report No.: VB1-AM000103MP1 MO-03-012003 Date: 14.08.2003 Non GLP, unpublished Confidential
II A, 4.1.3 /11	Hake, G.	2003d	Validaton of HPLC-method AM000303MP1, Fluoxastrobin Byproducts HPLC - External standard Bayer CropScience, Report No.: VB1-AM000303MP1 MO-03-012008 Date: 01.09.2003 Non GLP, unpublished Confidential
II A, 5.2.6 /02	Vohr, H.-W.	2003	HEC 5725-Study for the skin sensitization effect in guinea pigs (Guinea pig maximization test according to Magnusson and Kligman) Bayer CropScience AG, Report No.: AT00688, MO-03-12692 Date: 02.10.2003 GLP, unpublished
II A, 5.8.5/01	Herbold, B.	2002a	HEC 5725-E-CL-PMD - Salmonella/microsome test - Plate incorporation and preincubtion method Bayer CropScience AG, Report No.: AT00142, MO-02-018550 Date: 09.12.2002 GLP, unpublished
II A, 5.8.5/02	Kroetlinger, F.	2002a	HEC 5725-E-CL-PMD - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: AT00074, MO-02-016354 Date: 30.10.2002 GLP, unpublished
II A, 5.8.5/03	Herbold, B.	2002b	HEC 5725-Bisphenoxypyrimidine - Salmonella/microsome test - Plate incorporation and reincubation method Bayer CropScience AG, Report No.: AT00170, MO-03-000023 Date: 16.12.2002 GLP, unpublished
II A, 5.8.5/04	Kroetlinger, F.	2003a	HEC 5725-Bisphenoxypyrimidine - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: AT00200, MO-03-000573 Date: 13.01.2003 GLP, unpublished

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not
II A, 5.8.5/05	Herbold, B.	2002c	HEC 5725-DI-OH-PMD-BIS-E-DES-PMD - Salmonella/microsome test - Plate incorporation and preincubation method Bayer CropScience AG, Report No.: AT00153, MO-03-000017 Date: 12.12.2002 GLP, unpublished
II A, 5.8.5/06	Kroetlinger, F.	2002b	HEC 5725-DI-OH-PMD-BIS-E-DES-PMD - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: AT00038, MO-02-015651 Date: 18.10.2002 GLP, unpublished
II A, 5.8.5/07	Herbold, B.	2002d	HEC 5725-Des-fluoro - Salmonella/micrsome test - Plate incorporation and preincubation method Bayer CropScience AG, Report No.: AT00169, MO-03-000011 Date: 16.12.2002 GLP, unpublished
II A, 5.8.5/08	Kroetlinger, F.	2003b	HEC 5725-Des-Fluoro - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: AT00199, MO-03-000530 Date: 13.01.2003 GLP, unpublished
II A, 5.8.5/09	Herbold, B.	2003a	HEC 5725-Bis-phenoxy - Salmonella/microsome test - Plate incorporation and preincubation method Bayer CropScience AG, Report No.: AT00314, MO-03-002775 Date: 12.03.2003 GLP, unpublished
II A, 5.8.5/10	Kroetlinger, F.	2003c	HEC 5725-Bis-Phenoxy - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: AT00198, MO-03-000551 Date: 13.01.2003 GLP, unpublished
II A, 5.8.5/11	Herbold, B.	2002e	HEC 5725-Phenoxy-CF-pyrimidine - Salmonella/microsome test - Plate incorporation and preincubation method Bayer CropScience AG, Report No.: 31761, MO-02-002544 Date: 11.02.2002 GLP, unpublished
II A, 5.8.5/12	Kroetlinger, F.	2002c	HEC 5725-Phenoxy-CF-Pyrimidine - Study for acute oral toxicity in rats Bayer CropScience AG, Report No.: 31647, MO-02-000356 Date: 08.01.2002 GLP, unpublished

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not
	Heimann, K.G.	2003	Position paper concerning the reduction of serum ALT and AST levels
II A, 5.8.5/13	Herbold, B.	2003b	E-des-chlorophenyl - Project HEC 5725 - Salmonella/microsome test - Plate incorporation and preincubation method Bayer CropScience AG, Report No.: AT00679, Date: 01.10.2003 GLP, unpublished
II A, 6.0.1 /03	Heinemann, O.	2003	30 months storage stability of residues of HEC5725 during frozen storage in/on matrices of plant origin Bayer CropScience, Report No.: MR-563/01, Date: 04.03.2003, GLP, unpublished
II A, 8.2.4 /07	van Wijngaarden, R.P.A	2003	Acute toxicity of fluoxastrobin to freshwater invertebrates Bayer CropScience, Report No.: Alt.RW.2003.1 MO-03-007803 Date: 26.06.2003 GLP, unpublished
II A, 8.2.4 /08	Liebig, M	2003	A long-term indoor microcosm study on the toxicity ofFluoxastrobin (EC 100) to the Amphipod Gammarus pulex L, in a natural water sediment system Bayer CropScience, Report No.: P1MG GLP, unpublished
II A, 8.2.4 /09	Breuer, P and Heimbach, F	2003	Risk Assessment for the toxicity of fluoxastrobin to freshwater invertebrate organisms based on studies with additional non-standard freshwater invertebrate species. Not GLP, unpublished

Council Directive 91/414/EEC



Fluoxastrobin

Volume 4
(confidential)

Addendum 2 to the
Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

Draft: April 2004

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16508/EPCO/BVL/04

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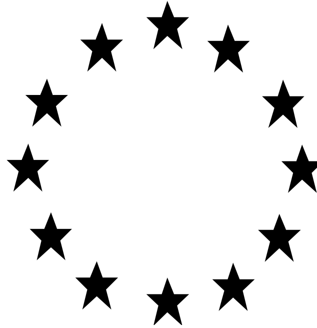
Council Directive 91/414/EEC



Fluoxastrobin

**Addendum 3 to the
Report and Proposed Decision of the United Kingdom made to
the European Commission under Article 8(1) of
91/414/EEC**

12 August 2004

PHYSICAL AND CHEMICAL PROPERTIES OF THE FORMULATION (ANNEX POINT IIIA 2.8): EFFECTIVENESS OF COMMERCIALLY AVAILABLE ANTIFOAM IN CONTROLLING THE FOAMING OF THE EC 100 FORMULATION

6929/EPCO/BVL/04

PHYSICAL AND CHEMICAL PROPERTIES OF THE FORMULATION: EFFECTIVENESS OF COMMERCIALLY AVAILABLE ANTIFOAM IN CONTROLLING THE FOAMING OF THE EC 100 FORMULATION

Background

In the Draft Assessment Report for Fluoxastrobin (B.2.2.17), persistent foaming (Point IIIA 2.8), according to CIPAC Method MT 47.2 was 125 ml after 1 minute. The limit for this test is 60 ml, therefore the potential risk to operators was considered. In the operator exposure section of the Draft Assessment Report (B.6.14), the RMS concluded that the use of an antifoam was necessary. The notifier had submitted a non-standard and non-GLP study that indicated that such an antifoam would be effective. Therefore, the RMS proposed that the effectiveness of an antifoam should be confirmed at the member state / product authorisation level. However, at the Evaluation Meeting held on 25 May 2004, member states considered that these data were needed before fluoxastrobin could be considered for inclusion in Annex I of Directive 91/414/EEC (Data requirement 1.1 of the Evaluation table, Point 1(18) of the Reporting Table). The notifier has submitted an appropriate study and this is evaluated below.

Evaluation

A GLP study in accordance with CIPAC MT 47.2 was performed. A 1% preparation* of the formulation (with and without the polydimethylsiloxane antifoam DOW AgroScience Schaumstopp, 0.0014%) was made up in CIPAC D water and the mixture was inverted 30 times. The results were as follows

<u>Persistent foaming of fluoxastrobin EC 100 with and without an antifoaming agent</u>

Time	Without antifoam (ml of foam)	With antifoam (ml of foam)
After 10 seconds	100.5 ml	12 ml
After 1 minute	94 ml	0 ml
After 3 minutes	77.5 ml	0 ml
After 12 minutes	48 ml	0 ml

(*) The "in-use" dilution of the formulation HEC 5725 EC 100 is 2 litres of formulation in 200 – 300 litres of water)

(Guildner 2004)

Conclusion

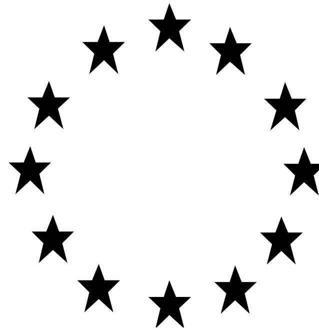
The data show that the antifoam DOW AgroScience Schaumstopp is effective in limiting the amount of foaming in the first instance and prevents the persistence of the foam.

List of studies which were submitted during the evaluation process and were not cited in the draft assessment report.

Annex point/ reference number	Author(s)	Year	Title Source (where different from company) Company name, Report No., Date, GLP status (where relevant), published or not

IIIA, 2.8.2	Guldner, W	2004	Persistent foaming of fluoxastrobin EC 100. Bayer CropScience, Study No. 1050 5312 GLP Not published.

Council Directive 91/414/EEC



Fluoxastrobin

Addendum 4 to the
Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

August 2004

Evaluation of supplementary histopathology data on the thymus of pups from two-generation reproductive toxicity study with fluoxastrobin in the Wistar rat. (Open Point 2.3 of the Evaluation Table for fluoxastrobin).

Evaluation of supplementary histopathology data on the thymus of pups from two-generation reproductive toxicity study with fluoxastrobin in the Wistar rat

(Open Point 2.3 of the Evaluation Table for fluoxastrobin).

Background

In the multigeneration study in rats with fluoxastrobin (Young and Christenson, 2001; see B.6.6.1 of the DAR), it is noted that from pups sacrificed on day 21, the brain, spleen, and thymus was weighed from one male and one female/litter if available. The RMS drew attention to the following organ weight changes in the **thymus of pups**.

> *Thymus: at 10,000 ppm, absolute thymus weight was decreased (by 35% for F1 pups, by 40% for F2 pups). It would appear that these decreases were not simply a reflection of reduced body weight (which was decreased by 21-26%) because thymus weight relative to body weight was also decreased statistically significantly (by 13% for F1 pups, by 24% for F2 pups).*
>
> *At 1,000 ppm in F2 pups, there was no decrease in mean body weight but thymus weight (absolute and relative to body weight) was decreased by 13%. The decrease in relative thymus weight was statistically significant.*
>
> *At 100 ppm in F2 pups, thymus weight (absolute and relative to body weight) was only 5-9% below controls and the differences were not statistically significant. The effect at this dose level is considered to be not toxicologically significant but it is supportive of a dose response.*

The applicant has commented that

> *"Due to the size of the thymus in a 21-day-old pup, there is a significant animal-to-animal variation in weight, due not only to normal variation, but also due to the excision and trimming of such a tiny organ. The thymic weights in the control animals ranged from .029 - .340 g in the male, and .092 - .376 g in the female. Moreover, the standard guideline procedure in place during the execution of this study was to necropsy only one male and one female per litter (if one of each sex was available), inadvertently contributing additional variability due to random selection based on sex and not body weight. Hence the applicant concludes that only the changes at 10,000 ppm are clearly of a magnitude consistent with exposure to the test substance. In addition, at the time this study was conducted, histopathology was not required on pups.*

The RMS agrees that the applicant's case is plausible. However, the presence of a clear dose-related reduction in mean relative thymus weight in F2 females, with statistically significant differences at 1,000 and 10,000 ppm, does suggest a true substance related effect (see Table B.6.27a).

Table B.6.27a: Thymus weight, absolute and relative to body weight, in pups from the multigeneration study: mean (and standard error), n= 23-28/sex/dose/generation

		0 ppm	*100 ppm*	*1000 ppm*	*10,000 ppm*
F1 generation					
Absolute	*Both sexes*	*0.204 (0.0064)*	*0.202 (0.0093)*	*0.194 (0.0077)*	*0.132** (0.0050)*
	Males	*0.206 (0.0072)*	*0.201 (0.0110)*	*0.192 (0.0100)*	*0.134** (0.0053)*
	Females	*0.203 (0.0069)*	*0.201 (0.0099)*	*0.196 (0.0073)*	*0.130** (0.0065)*
Relative	*Both sexes*	*0.449 (0.0136)*	*0.454 (0.0164)*	*0.430 (0.0129)*	*0.389** (0.0117)*
	Males	*0.443 (0.0145)*	*0.437 (0.0176)*	*0.417 (0.0172)*	*0.386* (0.0126)*
	Females	*0.455 (0.0153)*	*0.461 (0.0183)*	*0.440 (0.0125)*	*0.396* (0.0167)*
F2 generation					
Absolute	*Both sexes*	*0.207 (0.0104)*	*0.197 (0.0075)*	*0.181 (0.0063)*	*0.125** (0.0079)*
	Males	*0.203 (0.0111)*	*0.194 (0.0087)*	*0.183 (0.0074)*	*0.122** (0.0078)*
	Females	*0.211 (0.0120)*	*0.200 (0.0089)*	*0.179 (0.0076)*	*0.124** (0.0080)*
Relative	*Both sexes*	*0.463 (0.0156)*	*0.420 (0.0121)*	*0.401** (0.0113)*	*0.350** (0.0150)*
	Males	*0.444 (0.0200)*	*0.403 (0.0131)*	*0.404 (0.0126)*	*0.339** (0.0151)*
	Females	*0.480 (0.0181)*	*0.437 (0.0165)*	*0.402** (0.0151)*	*0.361** (0.0184)*

** P< = 0.05, ** p< = 0.01 (Dunnett's test)*

Based on these thymus findings for pups, the RMS proposed the following NOAEL for developmental effects:

> ***The NOAEL for developmental effects** is considered to be 100 ppm (16 mg/kg bw/day in lactating dams) based on slightly reduced thymus weight of pups at 1,000 ppm (171 mg/kg bw/day in lactating dams). To base the NOAEL on just one slight effect is probably conservative. However this precautionary approach is considered to be justified because the effect was clearly part of a dose response extending down to 100 ppm, there was no effect on body weight of the 1,000 ppm pups, no histopathological investigation of the pup thymus was performed and the consequence for subsequent development of these pups has not been investigated.*

The RMS did acknowledge in the DAR that:

> *The applicant however proposed a <u>higher NOAEL</u> (1,000 ppm) for developmental effects. This was based on effects including reduced body weight gain, delayed development and reduced weight of thymus and spleen of pups at 10,000 ppm (1,625 mg/kg bw/day in lactating dams).*

In order to support a NOAEL of 1000 ppm for developmental effects, the applicant has now submitted histopathology data for the thymus of F2 pups. These data have been provided as a supplementary submission (by Young 2004) to the original study report by Young and Christenson (2001).

In this addendum the RMS has evaluated the supplementary submission from the applicant.

Evaluation

This supplementary submission was conducted to GLP.

A histological investigation was conducted on the thymus of F2 pups from the control and 1,000 ppm dose groups. The histological techniques used are not described but they are assumed to involve the use of standard techniques (ie staining with H and E) as described for other histological investigations in the main study report.

The number of pups for which the thymus was investigated histologically was:

Control males: 28
Control females: 25
1000 ppm males: 25
1000 ppm females: 24

These pups were mostly the same pups as those whose thyroid gland was weighed in the main study (there were only 1 or 2 pups/sex/dose group that did not correspond between organ weight and histological investigations).

Each pup investigated in the 1000 ppm group is reported to have a “normal “ thymus, ie to show no morphological differences from the controls. It is also noted that generally, there was no obvious (visual comparison) reduction in size of tissue presented on the slide when comparing thymic tissue from 1000 ppm and control animals.

As no substance-related lesions were seen on histological examination of the thymus of F2 pups at 1000 ppm, the RMS considers that the slight reduction in absolute and relative thymus weight of F2 pups (by 13% for both sexes combined, by 15-16% for females) should be regarded as a possibly substance-related finding but not as an adverse effect. The lack of information on the subsequent development of these pups is now not considered to be a critical uncertainty.

The NOAEL for developmental effects in this study should therefore be revised as follows:

The NOAEL for developmental effects is considered to be 1000 ppm (171 mg/kg bw/day in lactating dams) based on the following effects in pups at 10,000 ppm (1625 mg/kg bw/day in lactating dams): reduced body weight gain, delayed development (delay in preputial separation), and reduced weight of thymus and spleen.

This NOAEL is the same as proposed by the applicant.

Conclusion

The additional histological investigation has provided sufficient evidence to support raising the NOAEL for developmental effects in the rat multigeneration study to 1000 ppm, which is line with the applicant's proposal.

Raising this NOAEL for developmental effects has no impact on the ADI, AOEL or ARfD which are all set based on adverse effects in dogs.

Fluoxastrobin

Addendum

Prepared by EFSA on 11 May, 2005

Section Ecotoxicology

B.9.1. Risk to birds.

In the DAR the risk to insectivorous and herbivorous birds was calculated by the RMS according to EPPO (1992). Therefore EFSA recalculated the Toxicity Exposure Ratio (TER) and Estimated Theoretical Exposure (ETE) based on the methods, i.e. equations and tabulated values described in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25. September 2002). The risk was calculated for the cereal scenario as proposed in the DAR. The endpoints are taken over from the original DAR.In addition the risk is calculated for a granivorous bird as one of the representative uses is a seed treatment.

Table 9.1-1: Acute, short-term and long term risk for birds

Type	Bird type	AR (kg/ha)	FIR/bw	RUD	Ftwa	MAF	ETE	Endpoint (mg/kg bw)	TER	Annex VI trigger
Acute	Herbivorous	0.2	0.44	142	-	1.2	14.995	2000	133.38	10
Acute	Insectivorous		1.04	52	-	-	10.816	2000	184.91	10
Short-term	Herbivorous		0.44	76	-	1.4	9.36	966	103.17	10
Short-term	Insectivorous		1.04	29	-	-	6.03	966	160.15	10
Long-term	Herbivorous		0.44	76	0.53	1.4	4.96	51	10.27	5
Long-term	Insectivorous		1.04	29	-	-	6.03	51	8.45	5
Acute	Granivorous	75 mg a.s./kg seed	0.38	-	-	-	28.5	2000	70.18	10
Short term	Granivorous		0.38	-	-	-	28.5	966	33.89	10
Long-term	Granivorous		0.38	-	-	-	28.5	51	**1.79**	5

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; ETE = Estimated theoretical exposure; TER = Toxicity exposure ratio.

The acute, short and long term risk for insectivorous and herbivorous birds from the representative use in cereals as a spray application can be regarded as low as the appropriate Annex VI trigger values were not breached. Also the acute and short term risk to granivorous birds can be regarded as low. The Annex VI trigger value for the long term risk to granivorous birds is breached indicating a high long term risk to granivorous birds in the first tier risk assessment. Therefore EFSA proposes a data requirement for the notifier to submit a refinement of the long term risk to granivorous birds if the risk is calculated according to the latest guidance document (SANCO/4145/2000).

B.9.3 Risk to mammals

In the DAR the risk to mammals was calculated by the RMS according to EPPO (1992). Therefore EFSA recalculated the Toxicity Exposure Ratio (TER) and Estimated Theoretical Exposure (ETE) based on the methods, i.e. equations and tabulated values described in the "Guidance Document on Risk Assessment for Birds and Mammals Under Council Directive 91/414/EEC" (Sanco/4145/2000 of 25. September 2002). The risk was calculated for the cereal scenario as proposed in the DAR. The endpoints are taken over from the original DAR. In addition the risk is calculated for a granivorous mammal as one of the representative uses is a seed treatment.

Table 9.3.2-1: Risk assessment for effects of fluoxastrobin on mammals

Type	Species	AR (kg/ha)	FIR/bw	RUD	Ftwa	MAF	ETE	Endpoint (mg/kg bw)	TER	Annex VI trigger
Acute	Small herbivorous mammal	0.2	1.39	142	-	1.2	47.37	2000	42.22	10
Acute	Insectivorous mammal		0.63	14	-	-	1.76		1133.79	10
Long-term	Small herbivorous mammal		1.39	76	0.53	1.4	15.68	742	47.33	5
Long-term	Insectivorous mammal		0.63	5.1	-	-	0.64		1154.68	5
Acute	Granivorous mammals	75 mg a.s./kg seed	0.23	-	-	-	17.25	2000	115.94	10
Long-term	Granivorous mammals		0.23	-	-	-	17.25	742	43.01	5

FIR = Food intake rate; bw = Bodyweight; RUD = Residue per unit dose; Ftwa = Time weighted average factor; AR = Application rate; ETE = Estimated theoretical exposure; TER = Toxicity exposure ratio.

The resulting acute and long term TER values are above the respective Annex VI trigger values, indicating a low risk to herbivorous, insectivorous and granivorous mammals from the representative uses of fluoxastrobin in cereals.

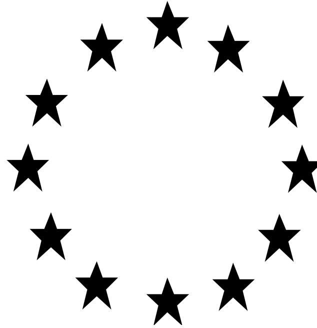
B.9.5 Risk to earthworms and B.9.6 Risk to other soil non-target organism

It was noted by EFSA that the RMS revised the PECsoil values for the metabolite M48 after the EPCO Expert meeting and hence also the corresponding TER values for earthworms and soil macro-organisms in the List of endpoints. EFSA agrees with the revised PECsoil calculations for the metabolite M48. For reasons of transparency the calculation leading to these revised TER values are given here by EFSA.

Species & study type	Test substance	Ecological endpoint	Soil PEC (mg/kg)	TER
Earthworms, 14 day acute study	HEC 5725-deschlorophenyl	LC50 > 1000 mg metabolite / kg dry soil	0.0149	67114
Earthworms, 56 day chronic study	HEC 5725-deschlorophenyl	NOEC = 1000 mg metabolite / kg dry soil	0.0149	67114
Folsomia candida: 28 day chronic study	HEC 5725-deschlorophenyl	NOEC = 100 mg metabolite / kg dry soil	0.0149	6711

The risk to earthworms and Folsomia candida from the metabolite M48 can be considered as low.

Council Directive 91/414/EEC



Fluoxastrobin

Addendum 5 to the
Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

June 2005

Clarification on the derivation of the AOEL for fluoxastrobin.
(Open point 2.5 of the Evaluation Table for fluoxastrobin)

Clarification on the derivation of the AOEL for fluoxastrobin.

Original proposal from the RMS as presented in the Draft Assessment Report.

Short-term systemic AOEL

In the case of the foliar-spray product 'HEC 5725 EC 100', the RMS notes that it is expected that application by individual growers would be restricted to a few days per annum, whereas contract operators might use the product for a few consecutive weeks. In both situations, operators will only experience short-term exposure (< 90 days/year). It is therefore considered appropriate to use a short-term AOEL for operator risk assessment of this product, and to base this AOEL on toxicology data derived from experiments of up to 90 days' duration.

The dog was shown to be the most sensitive species following exposure to fluoxastrobin. An overall 90-day NOAEL in dogs is 100 ppm (3 mg/kg bw/day) based on increased serum alkaline phosphatase in both sexes at 250 ppm (8 mg/kg bw/day) after 87 days in the 1-year dog study, see Section B.6.3.6 of the DAR. The NOAEL of 100 ppm was obtained in the first 90-day dog study and is also supported by effects (reduced body weight gain, reduced hepatic function, increased serum alkaline phosphatase) at 800 ppm (24-25 mg/kg bw/day) in the first 90-day dog study.

Oral absorption at a relevant dose level (1 mg/kg bw/day) has been shown to be extensive in rats (80-92% within 24-30h, see B.6.1.4 of the DAR). Hence no adjustment for oral absorption is necessary when setting a systemic AOEL. Also although there is evidence for extensive first pass metabolism, with extensive biliary excretion, no correction to account for the resultant possible limited systemic exposure is necessary. This is because many of the toxicological effects relate to the liver and gastrointestinal tract (reduced absorption of phosphorus) and because there was evidence of enterohepatic circulation.

A short-term systemic AOEL of 0.03 mg/kg bw/day is proposed for fluoxastrobin based on applying a 100-fold assessment factor to the NOAEL of 3 mg/kg bw/day determined in the first 90-day dog study (Jones and Elcock 2001). This NOAEL is based primarily on an effect (increased serum alkaline phosphatase) seen at 8 mg/kg bw/day after 87 days in the one-year dog study (Jones and Hastings, 2002) and is supported by the LOAEL in the first 90-day dog study.

Discussion at EPCO 14

The proposal for the AOEL was based on the dog studies. The main effects in dog studies were decreased body weight gain and increased serum alkaline phosphatase, but the body weight response was not always consistent between studies. A table describing the body weight effects at different doses at 90 days in the 3 dog studies was in the DAR (Table B.6.20) and is appended to this addendum. Because of the variation in body weight gains in two 90 day studies and the 90 day time point in the 1 year dog study, overall the RMS considered the NOAEL to be 8 mg/kg bw/day for bw gain across these three studies at 90 days. However, as there was an increase in alkaline phosphatase at 8 mg/kg bw/day at 90 days (See Appendix 2) in the one year study, the overall NOAEL at 90 days in these 3 studies was considered by the RMS to be 3 mg/kg bw/day. The EPCO 14 meeting discussed the range of findings in the dog studies before agreeing the RMS proposal of **0.03 mg/kg bw/day for the AOEL**.

Appendix 1

Table B.6 20 Body weight effects in dog studies with fluoxastrobin: effects after one week and 3 months

(a) **Body weight gain by test group males compared to controls**

Dose ppm (mg/kg bw/d)*	Study (mean day 0 weight)	Difference in mean body weight gain relative to concurrent control			
		Day 0 to Day 7		Day 0 to Day 84 or 91	
		Absolute difference in bw (reduction in food consumpt.)	bw % control	Absolute difference in bw	bw % control
25 (0.7)	2nd 90-day (8.5 kg)	- 0.1 kg	- 16%	- 0.1kg	- 4%
25 (0.8)	1-year (9.2 kg)	+ 0.2 kg	+ 76%	+ 0.3 kg	+ 8%
50 (1.4)	2nd 90-day (8.3 kg)	- 0.2 kg	- 64%	- 0.5 kg	-15%
50 (1.7)	1-year (8.4 kg)	+ 0.4 kg	+ 136%	+ < 0.1 kg	+ 2%
100 (3)	Ist 90-day (7.1 kg)	+ 0.1 kg	+ 18%	- 0.6 kg	- 18%
250 (8)	1-year (8.8 kg)	+ <0.1 kg	+19%	+ 0.5 kg	+15%
800 (25)	Ist 90-day (7.9 kg)	**- 0.1 kg** (0.1 kg)	**- 26%**	**- 1.3 kg**	**- 35%**
1,200 (35)	1-year (8.6 kg)	**- 0.1 kg** (1.0 kg)	**- 65%**	**- 0.9 kg**	**-25%**
2,500/3,000 (76)	Ist 90-day (8.1 kg)	**- 1.0 kg** (1.0 kg)	**-289%**	**-1.5 kg**	**- 40%**

(b) **Body weight gain by test group females compared to controls**

Dose ppm (mg/kg bw/d)*	Study (mean day 0 weight)	Difference in mean body weight gain relative to concurrent control			
		Day 0 to Day 7		Day 0 to Day 84 or 91	
		Absolute difference in bw (reduction in food consumpt.)	bw % control	Absolute difference in bw	bw % control
25 (0.7)	2nd 90-day (6.9 kg)	+ <0.1 kg	+ 13%	- 0.5 kg	-18%
25 (0.7)	1-year (7.7 kg)	+ 0.2 kg	+ 83%	+ < 0.1 kg	+ 1%
50 (1.5)	2nd 90-day (7.2 kg)	+ 0.1 kg	+ 28%	+ <0.1 kg	+2%
50 (1.5)	1-year (7.4 kg)	+ 0.3 kg	+ 127 %	+ 0.2 kg	+ 9%
100 (3)	Ist 90-day (7.6 kg)	- 0.2 kg	- 30%	+ 0.9 kg	+39%
250 (8)	1-year (7.7 kg)	+ 0.1 kg	+ 34%	- 0.3 kg	-12%
800 (24)	Ist 90-day (7.0 kg)	**- 0.4 kg** (0.3 kg)	**- 78%**	**-1.0 kg**	**-46%**
1,200 (37)	1-year (7.5 kg)	**- 0.3 kg** (1.0 kg)	**- 136%**	**-1.2 kg**	**-45%**
2,500/3,000 (75)	Ist 90-day (7.1 kg)	**- 1.1 kg** (1.0 kg)	**-204%**	**-1.3 kg**	**-58%**

(c) **Body weight gain by controls**

Sex	Study	Mean day 0 weight	Weight gain Day 0 to 7	Weight gain Day 0 to 84 or 91
Male	Ist 90 day	7.5 kg	0.4 kg	3.6 kg
	2nd 90-day	7.5 kg	0.4 kg	3.4 kg
	I year	9.7 kg	0.3 kg	3.5 kg
Female	Ist 90 day	7.0 kg	0.5 kg	2.2 kg
	2nd 90-day	7.1 kg	0.3 kg	3.0 kg
	I year	7.8 kg	0.3 kg.	2.6 kg

Day 84 = 2nd 90-day study
Day 91= Ist 90-day study and 1-year study

- dose in mg/kg bw/d = mean for whole of study

Appendix 2

Fluoxastrobin: Further tabulation of existing Dog data discussed at EPCO 14

1 year dog study

Alkaline phosphatase 4/dose: mean U/l (SD) % increase compared to concurrent control

Anova + students t 2 sided * P < 5%

Males

	-7d	**87d**	**178d**	**273d**	**386d**
0 ppm	187 (19)	109 (14)	78 (11)	73 (15)	63 (15)
25 ppm 0.8 mg/kg bw	177 (27)	132 (24)	89 (22)	77 (25)	69 (23)
50 ppm 1.7 mg/kg bw	160 (19)	124 (21)	85 (15)	75 (23)	66 (24)
250 ppm 8 mg/kg bw	**152 (25)**	**171* (44) 57% incr**	**138* (38) 77% incr**	**134* (34) 84% incr**	**123* (40) 95% incr**
1200 ppm 37 mg/kg bw	**166 (22)**	**207 * (38) 90% incr**	**184* (57) 136% incr**	**171* (55) 134% incr**	**174 * (57) 176% incr**

Females

	-7d	**87d**	**178d**	**273d**	**386d**
0 ppm	166 (38)	118 (26)	80 (13)	79 (11)	78 (13)
25 ppm 0.7 mg/kg bw	187 (44)	133 (27)	86 (23)	85 (23)	84 (34)
50 ppm 1.5 mg/kg bw	182 (36)	128 (23)	101 (45)	80 (29)	96 (25)
250 ppm 8 mg/kg bw	**168 (45)**	**168* (26) 42% incr**	**130* (23) 63% incr**	**194* (103) 146% incr**	**128* (40) 64% incr**
1200 ppm 37 mg/kg bw	**171 (23)**	**299 * (51) 153% incr**	**280* (61) 250% incr**	**257* (86) 225% incr**	**268 * (133) 243% incr**

90 day dog study

Alkaline phosphatase 4/dose: mean U/l (SD) % increase compared to concurrent control

Anova + Dunnets t 2 sided * P < 5%

Males

	-2d	**29d**	**62d**	**91d**
0 ppm	182 (70)	158 (55)	127 (44)	112 (42)
100 ppm 3 mg/kg bw	150 (33)	138 (27)	127 (27)	117 (20)
800 ppm 25 mg/kg bw	200 (24)	271 (117)	259* (100) **incr**	308 (159)
1000 ppm 76 mg/kg bw	199 (63)	233 (72)	252 (72)	**346* (147) incr**

Females

	-2d	**29d**	**62d**	**91d**
0 ppm	175 (21)	183 (57)	144 (28)	135 (23)
100 ppm 3 mg/kg bw	189 (29)	178 (34)	154 (34)	146 (25)
800 ppm 24 mg/kg bw	187 (27)	235 (58)	245 (57)	335 (167)
1000 ppm 75 mg/kg bw	209 (38)	223 (41)	**345* (133) incr**	**461* (215) incr**

Study investigator concluded that alkaline phosphatase was increased in mid and high dose males and females and these values were also increased above historical control values for the laboratory.

RMS concluded alkaline phosphatase was increased in mid and high dose males and females. This can be seen from the extent to which the range of individual data overlaps with concurrent controls (little or no overlap at 2 and 3 months). There was no increase in alkaline phosphatase at 3 mg/kg bw/day.

2nd 90 day dog study

In this study with a top dose of 50 ppm (1.4 – 1.5 mg/kg bw/day), there were no toxicologically significant effects on clinical chemistry parameters.

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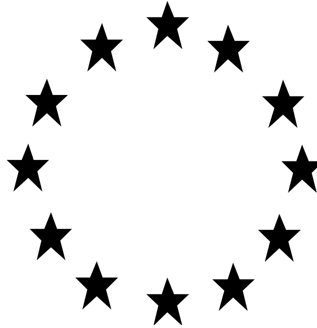


Fluoxastrobin

Addendum 6 to the
Report and Proposed Decision of the United Kingdom made to the European Commission under Article 8(1) of 91/414/EEC

June 2005

Risk to birds and mammals: Background information regarding the calculation of daily doses.
(Open point 5.3 of the Evaluation Table for fluoxastrobin)

Introduction

Exposure to birds to fluoxastrobin may result from consumption of either treated cereal foliage or of contaminated insects. There is a potential risk to birds from consumption of earthworms containing fluoxastrobin residues. However, this route of uptake is minor compared with that via consumption of insects, particularly as the proposed post-emergence use will result in only a proportion of the applied spray reaching the soil surface. Results of a fish bioaccumulation study indicated a whole fish bioconcentration factor of 52, which is less than the Annex VI trigger of 100.. Residues in fish are therefore unlikely to constitute a significant route of exposure and no further risk assessment for this was required. Therefore, the assessment made by the RMS related to the risk to large birds (>100g bw) from consumption of treated foliage and the risk to small birds (<100g bw) from the consumption of sprayed insects.

Residues of fluoxastrobin in contaminated insects and foliage were estimated in line with the EPPO (1992) vertebrate risk assessment scheme, based on estimates of initial residue values published by Hoerger & Kenaga (1982). For 'small insects' (which are only likely to be exposed to a single application), residue levels in mg/kg (or ppm) equals the dose contained in a single spray treatment in kg a.s. /ha (i.e. 0.2kg a.s. /ha) multiplied by the appropriate residue conversion factor (i.e. 29). For treated foliage, residue levels in mg/kg (or ppm) equals the maximum proposed total dose per crop in kg a.s. /ha (i.e. 0.4kg a.s. /ha) multiplied by the residue conversion factor for 'short grass' of 112 (this being representative of autumn / winter cereal grazing).

According to the EPPO vertebrate risk assessment scheme, the daily dry weight food consumption of a large bird (>100 g bw) is approximately equal to 10% of its bodyweight. Similarly the daily dry weight food consumption of a small bird (<100 g bw) is approximately equal to 30% of its bodyweight. This food intake was converted to wet weight using a factor of 2.4 which gave an intake equivalent to 24% (large bird) and 72% (small bird) of body weight. This was compatible with the estimated residue concentrations, which were in wet weight. These estimates were used together with the worse case assumption that the diet consisted entirely of contaminated food to calculate the acute and short-term dietary TERs that are presented in the list of end points.

As part of the Annex I peer-review process, the RMS was asked to make a risk assessment for birds and mammals available according to SANCO/4145/2000 using the present data available. However, the RMS noted that the dossier for fluoxastrobin was submitted in March 2002, significantly before the current Guidance document on risk assessment for birds and mammals was finalised. Given the relatively low toxicity to birds and mammals and that the calculated acute and long-term TERs are well within Annex VI triggers, it was unlikely that using the new guidance would significantly change the risk assessment, therefore it should not be necessary to repeat the risk assessment for birds and mammals. Following the EPCO expert ecotoxicology meeting the RMS was asked to supply background information regarding the calculation of daily doses. This is provided below

Background information regarding the calculation of daily dose values presented in the list of endpoints:

For birds the endpoints in the 5 day dietary and the 22 week reproductive toxicity studies have been converted from 'ppm a.s. in diet' to 'mg a.s./kg bw/day' as recommended under Section 2.2 of SANCO/4145/2000 (September 2002) guidance i.e. Daily dose in mg a.s./kg bw/day = (concentration in food mg/kg x daily food consumption g/bird/day) divided by body weight (g).

For mammals, the NOEL expressed in mg a.s. /kg bw/day was obtained directly from the Mammalian Toxicology section of the DAR (Section B.6.6.1).

Conversion to daily dose in 5-day Bobwhite quail dietary toxicity study:

5 day LC50 (oral) > 5000 ppm a.s. in diet.

Based on mean daily food consumption (average for 5000ppm treatment group over 5 day exposure period) of 5.6g food /bird /day and a mean body weight (for 5000ppm treatment group as average of 0 & 5 day exposure period body weights) of (23.2+34.8)/2 = 29.0 g.

Daily dose = 5000 x 5.6/29= 966 mg a.s./kg bw/day

Conversion to daily dose in 5-day Mallard duck dietary toxicity study:

5 day LC50 (oral) > 5000 ppm a.s. in diet.

Based on mean daily food consumption (average for 5000ppm treatment group over 5 day exposure period) of 49 g food /bird /day and a mean body weight (for 5000ppm treatment group as average of 0 & 5 day exposure period body weights) of (114.3+ 109.0)/2 = 111.65 g.
Daily dose = 5000 x 49/111.65 = 2194 mg a.s./kg bw/day

Conversion to daily dose in Bobwhite quail reproductive toxicity study:

NOEC = 1000 ppm a.s. in diet.

Based on mean daily food consumption (average for 1000ppm treatment group over 22 week exposure period) of 15.36 g food /bird /day and a mean body weight (for 1000ppm treatment group based on average of day 1 and day 155 values for both sexes) of 209 g (males) and 206.5 (females) = 207.75 g.

Daily dose = (1000 x 15.36)/207.75 = 74 mg a.s./kg bw/day

Conversion to daily dose in Mallard duck reproductive toxicity study:

NOEC = 461 ppm a.s. in diet.

Based on mean daily food consumption (average for 461 ppm treatment group over 20 week exposure period) of 124 g food /bird /day and a mean body weight (for 461 ppm treatment group based on average of week 1 and week 20 values for both sexes) of 1176.5 g (males) and 1065.5 g (females) = 1121.0 g.

Daily dose = (461 x 124)/1121 = 51 mg a.s./kg bw/day

The information above is summarised in the following table.

Species	**Study**	**Toxicity end point**	**Mean daily food consumption**	**Mean body weight**	**Daily dose (mg/kg bw/day)**
Bobwhite quail	5-day Dietary toxicity	LC50 > 5000 ppm	5.6 g food/ bird / day	29 g	966
Mallard duck	5-day Dietary toxicity	LC50 > 5000 ppm	49 g food/ bird / day	111.65 g	2194
Bobwhite quail	Reproductive	NOEC	15.36 g g food/	207.75 g	74

	toxicity	1000 ppm	bird / day		
Mallard duck	Reproductive toxicity	NOEC 461 ppm	124 g food/ bird / day	1121 g	51

Daily doses are presented in the list of end points.

Fluoxastrobin

Addendum

Prepared by EFSA on 26 July, 2005

EFSA conclusion on active substance Fluoxastrobin considers the metabolite 2-chlorophenol as relevant for the soil compartment (> 10 % of the applied quantity in soil). Since this metabolite has been relatively overlooked in the DAR and during the initial peer review (no comments from MS or EFSA in the reporting table), only in a late stage of the peer review some data gaps with respect to potential groundwater contamination and ecotoxicological tests have been identified. During Evaluation meeting (19-20, July 2005), RMS reiterated the case (already presented in the DAR) that due to the rapid degradation of 2-chlorophenol this metabolite would not accumulate. In this addendum, EFSA checked this case using the available information in order to assess if 2-chlorophenol may be expected to exceed the 10 % of applied quantity in soil. As a result of this assessment, **EFSA confirms that 2-chlorophenol must be considered a major aerobic metabolite of fluoxastrobin in soil**.

B.8.1 Route and rate of degradation is soil

B.8.1.4 Summary and assessment

Degradation of fluoxastrobin was studied in a number of laboratory and field studies summarized in the DAR. These studies showed that persistence of fluoxastrobin in soil may be very variable with half lives that range form 12 to 365 d in laboratory studies (mean DT_{50} = 54.2 d) and 16 to 119 d in field studies (mean DT_{50} = 39.1 d). No pH dependence or dependence to specific soil properties was clearly identified.
Cleavage of the clorophenyl moiety yields major metabolite M48 (max 32 % AR) and 2-chlorophenol. Actual amount of 2-chlorophenol formed was not measured in any of the available studies (chlorophenol ring was not labelled in the laboratory studies). The observed maximum amount of M48 suggests that 2-chlorophenol will be formed in amounts above 10 % (formation of 2-chlorophenol will be equimolar to the formation of M48). However, RMS argued in the DAR that 2-chlorophenol will not accumulate due to its fast degradation.
Degradation of 2-chlorophenol in soil is not addressed by any guideline study but with some studies published in the open scientific literature. These studies have been reassessed by EFSA to estimate soil DT_{50} of 2-chlorophenol based on the summaries provided in the DAR.

A-Baker and May, *Water, Air and Soil Pollution* 13 (1980) 411-424. (Summary in p 295 of the Fluoxastrobin DAR)

Laboratory study. Aerobic degradation. No half life is reported but it is stated that 70 % of 2-chlrorophenol or more is degraded in a minimum time of 0.5-1 d. In the worst case (1 d) this would correspond to a first order DT_{50} = 0.6 d (calculated by EFSA).

B-Alexander and Aleem, *Agricultural and Food Chemistry* (1961), vol 9, No 1, pp 44-47. (Summary in pp 295 and 296 of the Fluoxastrobin DAR)

Laboratory study. No half life is reported. Complete disappearance (i.e. 75-90 % decrease) was seen after 14 d for Dunkirk soil and after 47 d in the Mardin soil. This would correspond to DT_{50} between 4.37 d and 7.00 d for Dunkirk soil and between 14.69 d and 23 d for Mardin soil (calculated by EFSA).

C-Smith and Novak, *Water, Air and Soil Pollution* 33 (1987) 29-42. (Summary in p 296 of the Fluoxastrobin DAR)

Laboratory study. Degradation in subsurface saturated and unsaturated soils. Microcosm soil study mainly under anaerobic conditions. DT_{50} = 9 – 13 d are reported.

D- Crespin et al., *Environ. Sci. Technol.* (2001) 35, 4265-4270. (Summary in p 297 of the Fluoxastrobin DAR)

Field study. Andalucia, South of Spain. Degradation of 2,4-D and MCPA. No half life is reported for 2-chlorophenol as metabolite of 2,4-D and MCPA. Levels of 2-chlorophenol peaked after 5 and 11 d and decreased to below LOD after 15 d. Only 0-10 cm soil horizon was analyzed.

E- Walker, *Plant and Soil V*, no 2, February 1954. (Summary in pp 297 and 298 of the Fluoxastrobin DAR)

Laboratory soil percolation experiments. No half life provided but the study is not relevant to clarify the degradation in soil under aerobic conditions. Half life in the percolation soil was probably slightly below 10 d.

F- Van Gestel and Ma, Ecotoxicology and Environmetal Safety 15, 289-297 (1988). (Summary in p 298 of the Fluoxastrobin DAR)

Laboratory study. Toxicity study in earthworms were residue of 3-chlorophenol were measured. The paper gives DT_{50} = 2.1 d and 2.6 d at 15 ºC and DT_{50} = 2.5 d and 5.4 d at 23 ºC. No half life for 2-chlorophenol is measured in this paper. The RMS estimates that DT_{50} of 2-chlorophenol should be shorter than of 3-chlorophenol based on chemical structure.

G- Loehr and Matthews, *Journal of Soil Contamination*, 1(4): 339-360 (1992). (Summary in p 298 and 299 of the Fluoxastrobin DAR)

Laboratory study (laboratory batch microcosm). Two soils, one acidic soil Mississippi (pH 4.8) and a slightly alkaline soil Texas (pH 7.8). Conditions: aerobic, 20 °C, moisture content = 80 % field capacity. Poor recovery efficiencies (23 – 25 %) were obtained. A correction factor was applied to values actually measured to correct for this poor recovery efficiencies. Half lives were calculated as 7.2 d for the acidic soil and 1.7 d for the alkaline soil. RMS states that the paper also demonstrates that 2-chlorophenol degrades faster than 3-chlorophenol. EFSA is of the opinion that it is not

possible to derive any potential pH dependence on the degradation with only two soils; therefore the pH of the soils is disregarded with respect to further assessment.

Table B.8.4.1-1 Summarizes half lives taking into account only the values found in the literature for the degradation of 2-chlorophenol under aerobic conditions.

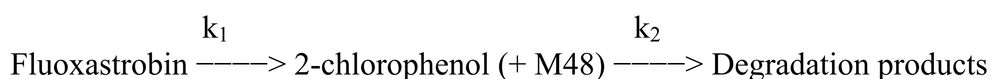
Table B.8.4.1-1 Half lives of 2-chlorophenol in soil under aerobic conditions.

Reference	A	B*		G		
Soil	A	Dunkirk	Mardin	Mississipi	Texas	**Mean**
DT_{50} (days)	0.6	7	23	7.2	1.7	7.9

* Worst case estimations reported

Estimation of the expected maximum amount of metabolite 2-chlorophenol in soil based on available data (EFSA Calculation).

To calculate the maximum amount of 2-chlorophenol that may be formed in soil the following degradation scheme has been assumed.



Formation and degradation of 2-chlorophenol has been simulated assuming the first order kinetics for both processes.
The differential equations that describe the above degradation scheme may be solved analytically. The equation that describes the formation and degradation of 2-chlorophenol may be written as in equation 1.

Equation 1:

$$[\text{2-chlrophenol}] = \frac{k_1\,[\text{fluoxastrobin}]_{ini}}{k_2 - k_1}\,[e^{k1t} - e^{k2t}]$$

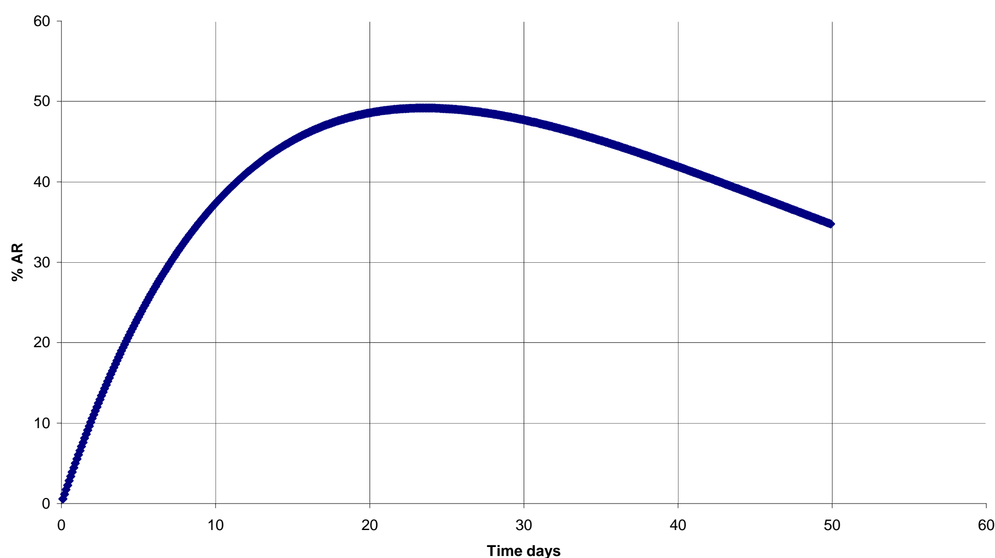
[2-chlrophenol]: actual concentration of metabolite 2-chlorophenol
$[\text{fluoxastrobin}]_{ini}$: initial concentration of fluoxastrobin

$k_1 = \ln(2) / DT_{50\ \text{fluoxastrobin}}$
$k_2 = \ln(2) / DT_{50\ \text{2-chlorophenol}}$

Worst case maximum formation of 2-chlorophenol will be produced when the faster degradation for fluoxastrobin is observed. Shortest laboratory aerobic DT_{50} of fluoxastrobin is 12 d that corresponds to a $k_1 = 0.058$. Worst case DT_{50} for 2-chlorophenol is 23 d (see above) that corresponds to a $k_2 = 0.03$

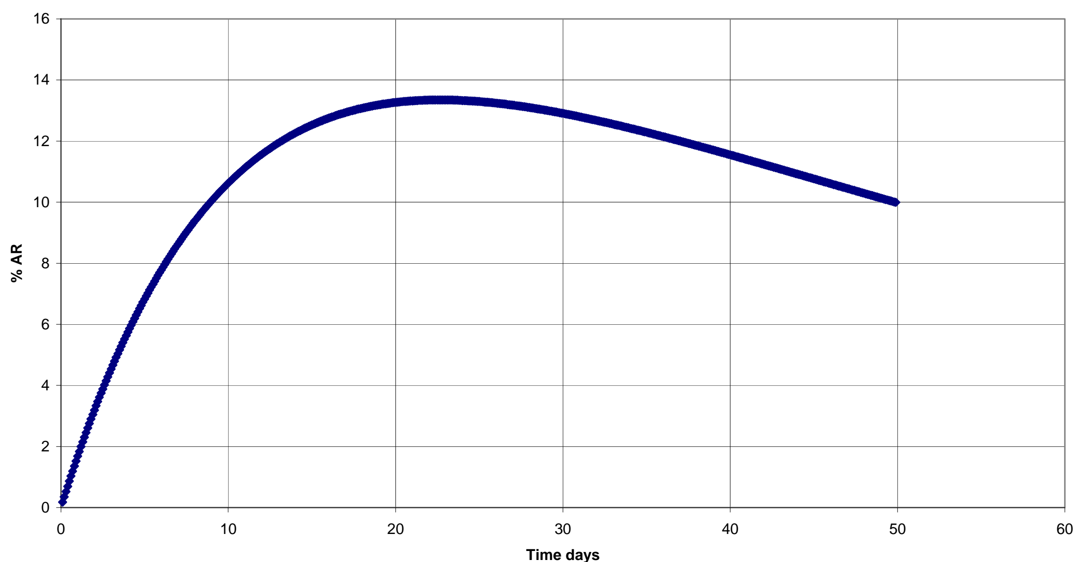
When equation 1 is represented a maximum amount of 2-chlorophenol corresponding to **49.18 % AR after 23.6 d** is obtained (see figure 1). **Therefore, 2-chlorophenol must be considered a major soil metabolite and should be assessed for potential ground water contamination and as a relevant soil metabolite by ecotoxicology.**

Figure 1



Whereas EFSA considers appropriate to use worst case assumptions with respect to the degradation of 2-chlorophenol (taking into account the limited information available and the uncertainties associated with the published studies) a calculation using mean DT_{50} = 7.8 d for 2-chlorophenol and mean DT_{50} = 39.1 d (value used in FOCUS gw calculation obtained from field studies) has also been performed. As a result of this calculation a maximum for 2-chlorophenol corresponding to 13.35 % AR after 22.7 d (see figure 2) has been obtained.

Figure 2



These calculations demonstrate that 2-chlorophenol may attain significant levels in soil, as a consequence of the degradation of fluoxastrobin, under a quite extensive number of situations and should be considered a relevant metabolite in soil with respect to the risk assessment.

B.8.3 Predicted environmental concentrations in soil (PECs) (IIIA 9.1)

Initial PECs based on the worst case maximum formation of 2-chlorophenol (49.18 %) and maximum initial PECs of fluoxastrobin (0.242 mg/kg) corrected for the molecular weight has been calculated by EFSA as: PECs ini (2-chlorophenol) = 0.033 mg / kg.